TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 USC 371

International Application No.: PCT/GB98/01627
International Filing Date: June 4, 19998
Priority Date Claimed: Yes
Title of Invention: VECTOR
Applicants for DO/EO/US: Susan Mary Kingsman, Robert Christopher, Fiona Margaret Ellard, Miles William Carroll, Kevin Alan Myers

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. (X) This is a **FIRST** submission of items concerning a filing under 35 USC 371.

2. (X) This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1).

3. (X) A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

4. (X) A copy of the International Application as filed (35 USC 371(c)(2))

   a) () is transmitted herewith (required only if not transmitted by the International Bureau).
   b) (X) has been transmitted by the International Bureau.
   c) () is not required, as the application was filed in the United States Receiving Office (RO/US).

5. (X) Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3))

   a) () are transmitted herewith (required only if not transmitted by the International Bureau).
   b) () have been transmitted by the International Bureau.
   c) () have not been made; however, the time limit for making such amendments has NOT expired.
   d) (X) have not been made and will not be made.

6. (X) A copy of the International Preliminary Examination Report with any annexes thereto, such as any amendments made under PCT Article 34.

7. (X) A copy of the International Search Report. For the Examiner's convenience, copies of the references cited therein are also included.

8. (X) A FIRST preliminary amendment.

9. (X) International Application as published.

10. (X) A Preliminary Amendment with attached paper copy of Sequence Listing.

11. (X) Computer-readable form of Sequence Listing.

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12. (X) A return prepaid postcard.

13. (X) The following fees are submitted:

				FEES
	BASIC FEE			$840
CLAIMS	**NUMBER FILED**	**NUMBER EXTRA**	**RATE**	
Basic Fee				$840
Total Claims	53 - 20 =	33 ×	$18	$594
Independent Claims	6 - 3 =	3 ×	$78	$234
Multiple dependent claims(s) (if applicable)			$260	$0
	TOTAL OF ABOVE CALCULATIONS			$1668
	TOTAL FEES ENCLOSED			$840

14. (X) The fee for later submission of the signed oath or declaration set forth in 37 CFR 1.492(e), as well as any excess claims fees, will be paid upon submission of the declaration.

15. (X) A check in the amount of $840 to cover the basic fee only is enclosed.

16. (X) The Commissioner is hereby authorized to charge only those additional fees which may be required to avoid abandonment of the application, or credit any overpayment to Deposit Account No. 11-1410. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Kingsman, et al.	) Group Art Unit Unknown
Int'l Appl. No.	:	PCT/GB98/01627	)
Int'l Filing Date	:	June 4, 1998	)
For	:	VECTOR	)
Examiner	:	Unknown	)

PRELIMINARY AMENDMENT
AND
SEQUENCE LISTING

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Preliminary to examination on the merits, please amend the above-identified U.S. National Phase Application as follows:

IN THE SPECIFICATION:

On page 1 of the Specification, after the Title of the Invention and before the Field of the Invention statement starting on line 3, please insert --This is the U.S. national Phase under 35 U.S.C. §371 of International Application PCT/GB98/01627, filed June 4, 1998, which claims priority of Great Britain application GB 9711579.4, filed June 4, 1997; GB 9713150.2, filed June 20, 1997 and GB 9714230.1, filed July 4, 1997.--.

On page 1 of the Specification, before the Field of the Invention statement, starting on line 3, please insert --FIELD OF THE INVENTION--.

On page 1 of the Specification after the Field of the Invention statement, please insert --BACKGROUND OF THE INVENTION--.

On page 2 at line 8 please insert --SUMMARY OF THE INVENTION--.

On page 7, line 7 please insert --DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS--.

On page 7, line 12, after the word "A" and before the word "immunoglobulin", please cancel the word "singly" and substitute in its place --single--.

On page 44, line 14, please insert --BRIEF DESCRIPTION OF THE DRAWINGS--.

On page 45, line 27, after the sequence "GTGTCCACTCC", please insert --(SEQ ID NO:23)--.

On page 53, line 16, after the sequence "5' GGG GGT GGT GGG AGC GGT GGT GGC GGC AGT GGC GGC GGC GGA A 3'" please insert --(SEQ ID NO:24)--.

On page 53, line 21, after the sequence "CC 3'" please insert --(SEQ ID NO:8)--.

On page 53, line 28, after the sequence "5' C TCG AAT TCC ACC ATG GCT TGC AAT TGT CAG TTG ATC C 3'" please insert --(SEQ ID NO:9)--.

On page 53, line 32, after the sequence "5' CTC CCC GGG CTT GCT ATC AGG AGG GTC TTC 3'" please insert --(SEQ ID NO:10)--.

On page 54, line 8, after the sequence "5' CTC ACT AGT GAG GTC CAG CTT CAG CAG TC 3'" please insert --(SEQ ID NO:11)--.

On page 54, line 12, after the sequence "5' CTC GCG GCC GCT TAC CGT TTG ATT TCC AGC TTG GTG CCT CCA CC 3'" please insert --(SEQ ID NO:12)--.

On page 56, line 11, after the sequence "ACA GCT ACA GGT GTC CAC TCC GAG GTC CAG ctgca" please insert --(SEQ ID NO:13)--.

On page 56, line 16, after the sequence "GAT GAT ACA GCT CCA TCC CAT GGTGGctcgagt" please insert --(SEQ ID NO:14)--.

On page 56, line 24, after the sequence "GTC CAG CTG CAG CAG TCT GG" please insert --(SEQ ID NO:15)--.

On page 56, line 28, after the sequence "CG TTT GAT TTC AAG CTT GGT GC" please insert --(SEQ ID NO:16)--.

On page 57, line 5, after the sequence "gcgc AAG CTT gaa atc aaa cgg GCC TCC ACC AAG GGC CCA" please insert --(SEQ ID NO:17)--.

On page 57, line 9, after the sequence "gcgc ctcgag TCA TTT ACC CGG AGA CAG GG" please insert --(SEQ ID NO:18)--.

On page 58, line 2, after the sequence "gcgc AAG CTT gaa atc aaa cgg GCC TCC ACA CAG AGC CCA" please insert --(SEQ ID NO:19)--.

On page 58, line 6, after the sequence "gcgc ctcgag TCA TTT ACC GGG ATT TAC AGA" please insert --(SEQ ID NO:20)--.

On page 59, line 7, after the sequence "GG ACT AGT AAT AGT GAC TCT GAA TGT CCC" please insert --(SEQ ID NO:21)--.

On page 59, line 11, after the sequence "ATT AGC GGC CGC TTA GCG CAG TTC CCA CCA CTT C" please insert --(SEQ ID NO:22)--.

On page 66, line 1, please cancel the word "CLAIMS" and substitute in its place --WHAT IS CLAIMED IS:--.

IN THE CLAIMS:

Cancel Claims 22, 23, 26, 30, 35, 39, and 44.

Amend the remaining claims as follows:

1. **(Amended)** A vector comprising a polynucleotide **[sequence ("NS")]** encoding **[for]** a **[tumour]**tumor-interacting protein **[("TIP") and optionally comprising a nucleotide sequence of interest ("NOI") which NOI encodes a product of interest ("POI");]** wherein the **[TIP]**tumor-interacting protein is capable of recognizing a **[tumour]**tumor, **[such that in use]** and wherein the vector is capable of delivering **[the NOI]** a second polynucleotide of interest **[and/or the POI]** to the **[tumour]**tumor.

2. **(Amended)** **[A]**The vector according to claim 1 wherein the vector comprises the **[NOI]** second polynucleotide of interest.

3. **(Amended)** **[A]**The vector according to claim 2 wherein the **[NOI]** second polynucleotide of interest is **[a]** therapeutic **[NOI and/or the POI is a therapeutic [POI] product of interest]**.

4. **(Amended)** **[A]**The vector according to **[any one of the preceding claims]**Claim 1 wherein **[in use]** the vector is capable of delivering the **[NOI]** second polynucleotide of interest **[and/or the POI]** to the interior of a **[tumour]**tumor mass.

5. **(Amended)** **[A]**The vector according to **[any one of the preceding claims]**Claim 1 wherein the **[TIP]**tumor-interacting protein **[is or]** comprises a **[tumour]**tumor-binding protein **[("TBP")]**.

6. [A]The vector according to **[any one of the preceding claims wherein the TIP is a TBP]**Claim 1 wherein the second polynucleotide of interest expresses a protein product of interest.

7. **(Amended)** **[A]**The vector according to **[any one of the preceding claims]**Claim 1 wherein the **[NS]**polynucleotide **[and/or the TIP]** comprises at least one **[tumour]**tumor binding domain capable of interacting with at least one **[tumour]**tumor-associated cell surface molecule **[("TACSM")]**.

8. **(Amended)** **[A]**The vector according to claim 7 wherein the **[TACSM]** tumor-associated cell surface molecule is selectively expressed on one cell type or on a restrictive number of cell types.

9. **(Amended)** **[A]**The vector according to **[any one of the preceding claims]**Claim 1 wherein **[in use]** the vector is capable of delivering the **[NOI]** second polynucleotide of interest **[and/or the POI]** to a selective **[tumour]**tumor site.

10. **(Amended)** **[A]**The vector according to **[any one of the preceding claims]**Claim 1 wherein the **[TIP]**tumor-interacting protein **[is or]** comprises at least part of an antibody.

11. **(Amended)** **[A]**The vector according to **[any one of the preceding claims]**Claim 1 wherein the **[TIP]**tumor-interacting protein **[recognises]**binds to a tropoblast cell surface antigen.

12. **(Amended)** **[A]**The vector according to claim 11 wherein the **[TIP recognises]**tropoblast cell surface antigen is the 5T4 antigen.

13. **(Amended)** **[A]**The vector according to **[any one of the preceding claims]**Claim 1 wherein the **[NS]**polynucleotide **[and NOI and/or the TIP]** and **[POI]** second polynucleotide of interest are **[linked together]**expressed as a fusion protein.

14. **(Amended)** **[A]**The vector according to claim **[13]**6 wherein the **[TIP]**tumor-interacting protein and **[POI]** product of interest are **[directly linked together]**expressed as a fusion protein.

15. **(Amended)** **[A]**The vector according to **[any one of the preceding claims]** Claim 1 wherein **[any one or more of]** any nucleotide sequence selected from the group consisting of : the **[NS, NOI, TIP]** polynucleotide encoding the tumor-interacting protein, the

second nucleotide sequence of interest, and both **[and the POI]** further comprises a polynucleotide sequence which encodes at least one additional functional component.

16. **(Amended)** **[A]**The vector according to **[any one of the preceding claims]**Claim 6 wherein any protein selected from the group consisting of: **[at least]** the **[TIP]**tumor-interacting protein, **[and/or POI]** the product of interest, and both, further comprises at least one additional functional component.

17. **(Amended)** **[A]**The vector according to claim 15 **[or 16]** wherein the additional functional component is selected from **[any one or more]**the group consisting of a **[signalling]**signaling entity **[(such as a signal peptide)]**, an immune enhancer, a toxin, **[or]**and a biologically active enzyme**[, or a sequence coding for any of same]**.

18. **(Amended)** **[A]**The vector according to **[any one of the preceding claims]**Claim 1 wherein the **[retroviral]** vector comprises a **[tumour specific promoter enhancer]**retroviral vector.

19. **(Amended)** **[A]**The vector according to **[any one of the preceding claims wherein the vector is a]**Claim 18 wherein the retroviral vector comprises a tumour specific promoter enhancer.

20. **(Amended)** A method of delivering a polynucleotide **[sequence]** of interest **[("NOI") and/]**or a product of interest **[("POI") encoded by same]**said polynucleotide of interest to a **[tumour, wherein]**tumor, comprising:

delivering the **[NOI]** polynucleotide of interest **[and/]**or **[POI]** product of interest **[are delivered]** to **[the tumour]**said tumor by use of **[a]**the vector of claim 1**[comprising the NOI and/or expressing the POI; wherein the NOI and/or the POI is capable of recognizing a tumour; wherein the NOI and/or the POI is delivered to the tumour; and wherein the vector is a vector according to any one of the preceding claims]**.

21. **(Amended)** **[A]**The method according to claim 20 wherein the vector is used to deliver the **[NOI]** polynucleotide of interest and**[/or POI]** product of interest *ex vivo* **[and/or *in vivo*]** to the **[tumour]**tumor.

24. **(Amended)** A method of treating **[a subject in need of same]**cancer in a mammal, the method comprising delivering a polynucleotide **[sequence]** of interest **[("NOI")]** **[and/]**or a product of interest **[("POI") encoded by same]** to a **[tumour]**tumor, wherein the **[NOI]** polynucleotide of interest **[and/]**or **[POI]** product of interest are delivered to the

[tumour]tumor by use of **[a vector comprising the NOI and/or expressing the POI; wherein the NOI and/or the POI is capable of recognising a tumour; wherein the NOI and/or the POI is delivered to the tumour; and wherein the vector is a]** the vector according to **[any one of the preceding claims]**claim 1.

25. **(Amended)** **[A]**The method according to claim 24 wherein the vector is used to deliver the **[NOI]** nucleotide sequence of interest **[and/]**or **[POI]** product of interest *ex vivo* **[and/or *in vivo*]**to the **[tumour]**tumor.

27. **(Amended)** A gene delivery system for targeting one or more genes encoding a **[TIP]**tumor-interacting protein **[(preferably a TBP)]** to a **[tumour]**tumor, comprising a genetic vector encoding a **[TIP]**tumor-interacting protein **[(preferably a TBP)]** and an *in vivo* gene-delivery system.

28. **(Amended)** A method of treating cancer comprising administering **[at least one TIP (preferably at least one TBP) gene to a]**the gene delivery system according to claim 27 **[either systemically or directly]** to the site of a **[tumour]**tumor.

29. **(Amended)** **[A]**The method **[gene delivery system for introducing one or more genes encoding a TIP (preferably a TBP) into cells]** of claim 28 wherein the tumor is of the haematopoietic **[(preferably myeloid haematopoietic)]** cell lineage **[either *in vivo* or *ex vivo*]**.

31. **(Amended)** A genetic vector comprising a **[therapeutic gene or genes]**polynucleotide encoding a **[TIP]**tumor-interacting protein **[(preferably a TBP),]** operably linked to an expression regulatory element selectively functional in a cell type present within a **[tumour]**tumor mass.

32. **(Amended)** **[A]**The genetic vector of claim 31 additionally comprising **[a therapeutic gene or genes is delivered to the interior of the tumour wherein the therapeutic gene encodes a TIP (preferably a TBP), which additionally contains]** one or more effector domains.

33. **(Amended)** A method of treating cancer in a mammal which comprises administering **[to an individual]** a combination of a cytokine or a cytokine-encoding gene and one or more **[TIP]**tumor-interacting protein **[(preferably a TBP)]** genes.

34. **(Amended)** **[The delivery of]**A method of delivering a gene to the site of a tumor comprising: **[TIP]**delivering tumor-interacting protein **[- (preferably a TBP-)]** encoding genes to the site of a **[tumour]**tumor.

36. **(Amended)** **[A]**The vector of Claim 14 wherein the **[comprising (a) a NS coding for a TIP and (b) an NOI which encodes a POI; wherein the TIP is capable of recognising a tumour such that in use the vector is capable of delivering the NOI and/or the POI to the tumour; wherein the TIP and POI are fused to each other; and wherein the POI]**fusion protein is capable of being secreted.

37. **(Amended)** **[Use of]**A method for producing a nucleotide sequence of interest comprising constructing the vector of Claim 1 and growing said vector in a compatible cell **[a vector according to any one of the preceding claims as an *in situ* production factory of any one or more of the NS, NOI, POI and TIP]**.

38. **(Amended)** **[Use of a vector according to any one of the preceding claims when]**A method for delivering a polynucleotide sequence to a second cell comprising placing a first cell containing the vector of claim 1 in close association with the second cell **[present in a cell to deliver any one or more of the NS, NOI, POI and TIP to a neighbouring cell]**.

40. **(Amended)** A process for preparing a **[TBP]**tumor-binding protein comprising expressing a **[NS]**polynucleotide encoding a **[TBP]**tumor-binding protein in a vector according to claim 1 **[4 or any claim dependent thereon]**.

41. **(Amended)** A **[TBP]**tumor-binding protein wherein the **[TBP]**tumor-binding protein is selected from a group consisting of: 5T4ScFv.1, 5T4Sab1, 5T4ScFv-IgG, 5T4ScFv-IgE1, B7-1.5T4.1, B7-1.5T4.2 and B7-EGF.

42. **(Amended)** A **[TBP]**tumor-binding protein obtained by the process of claim 40 **[or the TBP of claim 41 for subsequent use in a medical application]**.

43. **(Amended)** A method for diagnosis of cancer comprising: identifying or quantitating the tumor using the**[TBP]**tumor-binding protein according to claim 42 **[wherein the medical application is a diagnostic application]**.

45. **(Amended)** **[Use]**A method for the prognosis of cancer comprising: identifying and/or quantitating **[of]** a **[TACSM]**tumor-associated cell surface molecule **[as defined in claim 7 or claim 8 as a prognostic factor and/or a target for cancer therapy]**.

46. **(Amended) [Use of a TACSM according to]**The method of claim 45 wherein the **[TACSM]**tumor-associated cell surface molecule is erb-2.

Please add the following claims:

47. The vector according to Claim 1 wherein the vector is capable of delivering the protein product of interest to the interior of a tumor mass.

48. The vector of Claim 17 wherein the signaling entity is a signal peptide.

49. The method according to claim 20 wherein the vector is used to deliver the polynucleotide of interest and/or product of interest ***in vivo*** to the tumor.

50. The method according to claim 24 wherein the vector is used to deliver the nucleotide sequence of interest and/or product of interest ***in vivo*** to the tumor.

51. The gene delivery system of Claim 27 wherein the tumor-interacting protein is a TBP.

52. The method of Claim 28 wherein the gene delivery system is administered systemically.

53. The method of Claim 28 wherein the gene delivery system is administered directly to the site of the tumor.

54. The method of Claim 30 wherein the haematopoietic lineage is a myeloid lineage.

55. The method of Claim 30 wherein the gene delivery system is administered *in vivo*.

56. The method of Claim 30 wherein the gene delivery system is administered *ex vivo*.

57. The genetic vector of Claim 31 wherein the tumor-interacting protein is a tumor binding protein.

58. The method of Claim 33 wherein the tumor-interacting protein is a tumor binding protein.

59. The method of Claim 33 wherein the tumor-interacting protein is a tumor binding protein.

60. The vector of Claim 40 wherein the protein product of interest is therapeutic.

IN THE SEQUENCE LISTING

Please cancel from the application Original Sequence Listing pages 63-65 and substitute therefore the attached Replacement Sequence Listing pages 1-9. Please consecutively renumber all pages following the canceled Original Sequence Listing.

REMARKS

The claims have been amended to conform with the rules of practice before the Patent and Trademark Office. The specification has been amended to recite the International Application and to add 17 sequence identifiers and to correct minor informalities. As a result of this preliminary amendment, Claims 22, 23, 26, 30, 35, 39 and 44 have been canceled. Claims 47-60 have been added. Support for the added claims can be found in the original claims.

VERIFICATION UNDER 37 C.F.R. §1.821(f) & (g)

All of the sequences in the attached Sequence Listing were included in the application as filed. Pursuant to 37 C.F.R. §1.821(g), no new matter is being added herewith. As required under 37 C.F.R. §1.821(f), I hereby verify that the data on the enclosed disk and the paper copies of the Sequence Listing are identical.

Conclusion

Should there be any questions concerning this application, the Examiner is invited to contact the undersigned attorney at the telephone number appearing below.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 6 Dec. 1999

By: Daniel Altman

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VECTOR

The present invention relates to a vector, preferably for use in medicine.

As it is well known in the art, a vector is a tool that allows or faciliates the transfer of an entity from one environment to another. By way of example, some vectors used in recombinant DNA techniques allow entities - such as a segment of DNA (such as a heterologous DNA segment, such as a heterologous cDNA segment) - to be transferred into a target cell. Optionally, once within the target cell, the vector may then serve to maintain the heterologous DNA within the cell or may act as a unit of DNA replication. Examples of vectors used in recombinant DNA techniques include plasmids, chromosomes, artificial chromosomes or viruses.

Thus, vectors can be used to deliver proteins and/or nucleotide sequences to targeted cells, such as tumour cells.

However, as it is well known, nucleotide sequences and proteins are complex molecules which may be produced from biological sources, most usually from genetically engineered organisms or cell cultures. Furthermore, the procedures for the production of nucleotide sequences and proteins can be complicated, labour intensive and costly. Furthermore, pharmacological properties and other aspects of the function of some proteins - such as immunoglobulins derived from non-human biological sources - and nucleotide sequences may frequently differ in important ways from the activity of the corresponding natural human immunoglobulins produced in human cells. By way of background information, an immunoglobulin is a member of a family of related multimeric proteins which are normally secreted from cells of the B-lymphocyte lineage of a vertebrate, whose typical function is to bind specifically with a region of a macromolecule identified as non-self. Immunoglobulins represent a major component of the immune response repertoire of the organism and are synonymous with "antibodies".

One major cause of such differences in activity may be due to variations in the pattern of glycosylation of proteins derived from different species (reviewed in Bebbington 1995; In Monoclonal Antibodies: the second generation ed. H. Zola pg 165-181). Furthermore, systemic administration of proteins (especially those containing toxin domains) and nucleotide sequences can induce additional pharmacokinetic and toxicological problems (reviewed in Scheinberg and Chapman 1995. In Monoclonal antibodies (ed. Birch and Lennox) Chapter 2.1).

Thus, the present invention seeks to provide an improved vector system for delivering a nucleotide sequence of interest and/or a product expressed by the same.

According to a first aspect of the present invention there is provided a vector comprising a nucleotide sequence ("NS") coding for a tumour interacting protein ("TIP") and optionally comprising a nucleotide sequence of interest ("NOI") which NOI encodes a product of interest ("POI"); wherein the TIP is capable of recognising a tumour, such that in use the vector is capable of delivering the NOI and/or the POI to the tumour.

According to a second aspect of the present invention there is provided a method of delivering a nucleotide sequence of interest ("NOI") and/or a product of interest ("POI") encoded by same to a tumour, wherein the NOI and/or POI are delivered to the tumour by use of a vector comprising the NOI and/or expressing the POI; wherein the NOI and/or the POI is capable of recognising a tumour; wherein the NOI and/or the POI is delivered to the tumour; and wherein the vector is a vector according to the present invention.

According to a third aspect of the present invention there is provided the use of a vector to deliver a nucleotide sequence of interest ("NOI") and/or a product of interest ("POI") encoded by same to a tumour, wherein the NOI and/or POI are delivered to the tumour by use of the vector which comprises the NOI and/or expresses the POI; wherein the NOI and/or the POI is capable of recognising a tumour when the NOI and/or the POI is delivered to the tumour; and wherein the vector is a vector according to the present invention.

According to a fourth aspect of the present invention there is provided a method of treating a subject in need of same, the method comprising delivering a nucleotide sequence of interest ("NOI") and/or a product of interest ("POI") encoded by same to a tumour, wherein the NOI and/or POI are delivered to the tumour by use of a vector comprising the NOI and/or expressing the POI; wherein the NOI and/or the POI is capable of recognising a tumour; wherein the NOI and/or the POI is delivered to the tumour; and wherein the vector is a vector according to the present invention.

According to a fifth aspect of the present invention there is provided the use of a genetic vectors to deliver a therapeutic gene encoding a TIP - preferably a tumour binding protein ("TBP") - more preferably a secretable TIP (preferably a secretable TBP) - to the interior of a tumour mass.

According to a sixth aspect of the present invention there is provided a gene delivery system for targeting one or more genes encoding a TIP (preferably a TBP) to a tumour, comprising a genetic vector encoding a TIP (preferably a TBP) and an *in vivo* gene-delivery system.

According to a seventh aspect of the present invention there is provided a method of treating cancer comprising administering a TIP (preferably a TBP) gene or genes in a gene delivery system according to the present invention either systemically or directly to the site of a tumour.

According to an eighth aspect of the present invention there is provided a gene delivery system for introducing one or more genes encoding a TIP (preferably a TBP) into cells of the haematopoietic (preferably myeloid haematopoietic) cell lineage either *in vivo* or *ex vivo*.

According to a ninth aspect of the present invention there is provided a method for treating cancer in a mammal, comprising administering to an individual a gene delivery system according to the present invention that is capable of expressing a TBP in cells derived from a haematopoietic (preferably myeloid haematopoietic) origin.

According to a tenth aspect of the present invention there is provided a genetic vector comprising a therapeutic gene or genes encoding a TIP (preferably a TBP), operably linked to an expression regulatory element selectively functional in a cell type present within a tumour mass.

According to an eleventh aspect of the present invention there is provided a genetic vector comprising a therapeutic gene or genes is delivered to the interior of the tumour wherein the therapeutic gene encodes a TIP (preferably a TBP), which additionally contains one or more effector domains.

According to a twelfth aspect of the present invention there is provided a method of treating cancer in a mammal which comprises administering to an individual a combination of a cytokine or a cytokine-encoding gene and one or more TIP (preferably a TBP) genes according to any of the previous aspects of the invention.

According to a thirteenth aspect of the present invention there is provided the delivery of TIP- (preferably a TBP-) encoding genes to the site of a tumour.

Preferably the vector comprises the NOI.

In one preferred aspect, the vector is expressing the POI.

The vector of the present invention may be useful for *inter alia* medical applications - such as diagnostic or therapeutic applications.

Preferably the NOI is a therapeutic NOI and/or the POI is a therapeutic POI.

On occasions in the following text, the NS and NOI may be individually or collectively referred to as being a gene.

The NS and NOI can be any suitable nucleotide sequence. For example, independently

they can be DNA or RNA - which may synthetically prepared or may be prepared by use of recombinant DNA techniques or may be isolated from natural sources or may be combinations thereof. The NOI may be a sense sequence or an antisense sequence.

There may be a plurality of NSs or NOIs, which may be directly or indirectly joined to each other, or combinations thereof. Thus, the expressed product may have two or more effector domains (which may be the same or different) and/or two or more TIP domains (which may be the same or different).

Preferably in use the vector is capable of delivering the NOI and/or the POI to the interior of a tumour mass.

In addition to cancerous cell, the cell types present within a tumour mass include but are not limited to macrophages, lymphocytes, tumour infiltrating lymphocyes, endothelial cells etc.

Preferably the NS and/or the TIP comprises at least one tumour binding domain capable of interacting with at least one tumour associated cell surface molecule ("TACSM").

In accordance with the present invention the TACSM can include but is not limited to a cell surface molecule which plays a role in tumour cell growth, migration or metastasis, a receptor for adhesive proteins such as the integrin vitronectin receptor, a growth factor receptor (such as epidermal growth factor (EGF) receptor, platelet-derived growth factor (PDGF) receptor, fibroblast-derived growth factor (FDGF) receptor, nerve growth factor receptor, insulin-like growth factor (IGF-1) receptor; a plasminogen activator; a metalloproteinase (such as colllagenase) 5T4 antigen; a tumour specific carbohydrate moiety; an oncofetal antigen; a mucin; a growth factor receptor; a glycoprotein; and an antigen restricted in its tissue distribution.

Preferably the TACSM is selectively expressed on one cell type or on a restrictive number of cell types.

Preferably in use the vector is capable of delivering the NOI and/or the POI to a selective tumour site.

Preferably the TIP is or comprises a tomour binding protein ("TBP").

Preferably the TIP is a TBP.

Examples of a TBP include: an adhesion molecule such as Intercellular adhesion molecule, ICAM-1, ICAM-2, LFA-1, LFA-2, LFA-3, LECAM-1, VLA-4, ELAM, N-CAM, N-cadherin, P-Selectin, CD44 and its variant isoforms (in particular CD44v6, CD44v7-8), CD56; a growth factor receptor ligand such epidermal growth factor (EGF), Platelet-derived growth factor (PDGF), Fibroblast-derived growth factor (FDGF), Nerve growth factor, vasopressin, insulin, insulin-like growth factor (IGF-1), hepatocyte growth factor, nerve growth factor, human growth factor, brain derived growth factor, ciliary neutrophic factor, glial cell line-derived growth factor; heavy and light chain sequences from an immunoglobulin (Ig) variable region (from human and animal sources), engineered antibody or one from a phage display library. A phage display library is a technique of expressing immunoglobulin genes in bacteriophage has been developed as a means for obtaining antibodies with the desired binding specificities. Expression systems, based on bacteriophage lambda, and more recently filamentous phage have been developed. The bacteriophage expression systems can be designed to allow heavy and light chains to form random combinations which are tested for their ability to bind the desired antigen.

The TBP may contain an effector domain which is activated on binding of the TPB to the TASCM. The effector domain or momains may be activated on binding of the TBP to a TASCM leading to inhibition of tumour cell proliferation, survival or dissemination. The effector domain may possess enzymatic activity (such as a pro-drug activating enzyme) or the effector domain may include a toxin, or an immune enhancer, such as a cytokine/lymphokine such as those listed above.

Preferably the TBP comprises one or more binding domains capable of interacting with one or more TACSMs which are present on the cancerous cells - which TACSMs may be the

same or different.

The term "interacting" includes direct binding, leading to a biological effect as a result of such binding.

Preferably the TIP is or comprises at least part of an antibody.

As is well known, antibodies play a key role in the immune system. In brief, the immune system works in three fundamentally different ways: by humoral immunity, by cellular immunity and by secretion of stimulatory proteins, called lymphokines. Humoral immunity relies on proteins collectively called immunoglobulin which constitute about 20% of the proteins in the blood. A singly immunoglobulin molecule is called an antibody but "antibody" is also used to mean many different molecules all directed against the same target molecule. Humoral immunity also involves complement, a set of proteins that are activated to kill bacteria both nonspecifically and in conjunction with antibody.

In cellular immunity, intact cells are responsible for recognition and elimination reactions. The body's first line of defense is the recognition and killing of microorganisms by phagocytes, cells specialised for the ingestion and digestion of unwanted material. These cells include neutrophils and macrophages. A key role of antibodies is to help phagocytes recognise and destroy foreign materials.

In order to perform these functions, the antibody is divided into two regions: binding (Fab) domains that interact with the antigen and effector (Fc) domains that signal the initiation of prcesses such as phagocytosis. Each antibody molecule consists of two classes of polypeptide chains, light (L) chains and heavy (H) chains. A single antibody has two indentical copies of the L chain and two of the H chain. The N-terminal domain from each chain forms the variable regions, which constitute the antigen-binding sites. The C-terminal domain is called the constant region. The variable domains of the H (V_H) and L (V_L) chains consitute an Fv unit and can interact closely to form a single chain Fv (ScFv) unit. In most H chains, a hinge region is found. This hinge region is flexible and allows the Fab binding regions to move freely relative to the rest of the molecule. The hinge

region is also the place on the molecule most susceptible to the action of protease which can split the antibody into the antigen binding site (Fab) and the effector (Fc) region.

The domain structure of the antibody molecule is favourable to protein engineering, facilitating the exchange between molecules of functional domains carrying antigen-binding activities (Fabs and Fvs) or effector functions (Fc). The structure of the antibody also makes it easy to produce antibodies with an antigen recognition capacity joined to molecules such as toxins, lymphocytes or growth factors.

Monoclonal antibodies are homogenous antibodies of the same antigenic specificity representing the product of a single clone of antibody-producing cells. It was recognised that monoclonal antibodies offered the basis for human therapeutic products. However, although mouse antibodies are similiar to human antibodies, they are sufficiently different that they are recognised by the immune system as foreign bodies, thereby giving rise to an immunological response. This human-anti-mouse-antibody (HAMA) response limits the usefulness of mouse antibodies as human therapeutic products.

Chimeric antibody technology involves the transplantation of whole mouse antibody variable domains onto human antibody constant domains. Chimeric antibodies are less immunogenic than mouse antibodies but they retain their antibody specificity and show reduced HAMA responses.

In chimeric antibodies, the variable region remains completely murine. However, the structure of the antibody makes it possible to produce variable regions of comparable specificity which are predominantly human in origin. The antigen-combining site of an antibody is formed from the six complementarity-determining regions (CDRs) of the variable portion of the heavy and light chains. Each antibody domain consists of seven antiparallel β-sheets forming a β-barrel with loops connecting the β-strands. Among the loops are the CDR regions. It is feasible to more the CDRs and their associated specificity from one scaffolding β-barrel to another. This is called CDR-grafting. CDR-grafted antibodies appear in early clinincal studies not to be as strongly immunogenic as either mouse or chimaeric antibodies. Moreover, mutations may be made outside the CDR in

order to increase the binding activity thereof, as in so-called humanised antibodies.

Fab, Fv, and single chain Fv (ScFv) fragments with VH and VL joined by a polypeptide linker exhibit specificities and affinities for antigen similiar to the original monoclonal antibodies. The ScFv fusion proteins can be produced with a nonantibody molecule attached to either the amino or carboxy terminus. In these molecules, the Fv can be used for specific targeting of the attached molecule to a cell expressing the appropriate antigen. Bifunctional antibodies can also be created by engineering two different binding specificities into a single antibody chain. Bifunctional Fab, Fv and ScFv antibodies may comprise engineered domains such as CDR grafted or humanised domains.

In virally directed enzyme therapy (VDEPT), a foreign gene is delivered to normal and cancerous cells by a viral vector - such as a retroviral vector. The foreign gene codes for an enzyme that can convert a non-toxic prodrug (eg 5-fluorocytosine) to a toxic metabolite (5-fluorouracil) that will kill those cells making it (Sikora *et al* 1994 Ann New York Acad Sci 71b: 115-124). If the promoter utilised is tumour specific, then the toxic product will only be synthesised in the tumour cells. Studies in animal models have demonstrated that this type of treatment can deliver up to 50-fold more drug than by conventional means (Connors and Knox 1995 1995 Stem Cells 13: 501-511). A variation of this technique uses tumour associated antibodies conjugated to prodrug converting enzymes to provide specific delivery to tumours. This method is referred to as antibody-directed enzyme prodrug therapy (ADEPT) (Maulik S and Patel SD "Molecular Biotechnology" 1997 Wiley-Liss Inc pp 45).

A large number of monoclonal antibodies and immunoglobulin-like molecules are known which bind specifically to antigens present on the surfaces of particular cell types such as tumour cells. Procedures for identifying, characterising, cloning and engineering these molecules are well established, for example using hybridomas derived from mice or transgenic mice, phage-display libraries or scFv libraries. Genes encoding immunoglobulins or immunoglobulin-like molecules can be expressed in a variety of heterologous expression systems. Large glycosylated proteins including immunoglobulins are efficiently secreted and assembled from eukaryotic cells, particularly mammalian cells.

Small, non-glycosylated fragments such as Fab, Fv, or scFv fragments can be produced in functional form in mammalian cells or bacterial cells.

The immunoglobulin or immunoglobulin-like molecule may be derived from a human antibody or an engineered, humanised rodent antibody such as a CDR-grafted antibody or may be derived from a phage-display library or may be a synthetic immunoglobulin-like molecule.

The antigen-binding domain may be comprised of the heavy and light chains of an immunoglobulin, expressed from separate genes, or may use the light chain of an immunoglobulin and a truncated heavy chain to form a Fab or a $F(ab)'_2$ fragment. Alternatively, truncated forms of both heavy and light chains may be used which assemble to form a Fv fragment. An engineered scFv fragment may also be used, in which case, only a single gene is required to encode the antigen-binding domain. In one prefered aspect, the antigen-binding domain is formed from a Fv or a scFv.

When a pathogen invades the body, lymphocytes respond with three types of reaction. The lymphocytes of the humoral system (B cells) secrete antibodies that can bind to the pathogen, signalling its degradation by macrophages and other cells. The lymphocytes of the cellular system (T cells) carry out two major types of functions. Cytotoxic T lymphocytes (CTLs) develop the ability to directly recognise and kill the cells infected by the pathogen. Helper T cells (TH cells) independently recognise the pathogen and secrete protein factors (lymphokines) that stimulate growth and responsiveness of B cells, T cells, and macrophages, thus greatly strengthening the power of the immune response.

Thus, in one preferred aspect, the TIP comprises an immunoglobulin, or a part thereof, or a bioisostere thereof.

In a preferred embodiment, the TIP comprises IgG and/or IgE, or a part thereof, or a bioisostere thereof.

In a more preferred embodiment, the TIP comprises IgE, or a part thereof, or a bioisostere

thereof.

Preferably the TIP recognises a trophoblast cell surface antigen.

Preferably the TIP recognises the 5T4 antigen.

The trophoblast cell surface antigen, originally defined by monoclonal antibody 5T4 (Hole and Stern 1988 Br. J. Cancer 57; 239-246), is expressed at high levels on the cells of a wide variety of human carcinomas (Myers *et al.* 1994 J. Biol. Chem. 269; 9319-9324) but, in normal tissues of non-pregnant individuals, is essentially restricted to low level expression on a few specialised epithelia (Myers *et al. ibid.* and references therein). The 5T4 antigen has been implicated in contributing to the development of metastatic potential and therefore antibodies specifically recognising this molecule may have clinical relevance in the treatment of tumours expressing the antigen.

The variable region of the 5T4 monoclonal antibody can also be humanised by a number of techniques, which are known in the art, including grafting of the CDR region sequences on to a human backbone. These can then be used to construct an intact humanised antibody or a humanised single chain antibody (Sab), such as an ScFv coupled to an Fc region (see Antibody Engineering: a practical approach, ed McCafferty *et al.* 1996 OUP).

Here the term Sab is not limited to just a human or a humanised single chain antibody. Preferably, however the Sab is a human single chaim anitibody or a humanised single chain antibody, or part thereof - such as ScFv coupled to an Fc region.

Preferably the NS and NOI and/or the TIP and POI are linked together.

Preferably the TIP and POI are directly linked together.

Preferably any one or more of the NS, NOI, TIP, and POI further comprise at least one additional functional component.

Preferably, at least the TIP and/or POI further comprise at least one additional functional component.

Preferably the additional functional component is selected from any one or more of a signalling entity (such as a signal peptide), an immune enhancer, a toxin, or a biologically active enzyme.

In a preferred aspect the POI is a secretable POI. Thus, in this aspect of the present invention, preferably, the additional functional component is at least an entity capable of causing the POI to be secreted - such as a signalling entity.

Another preferred additional component is a promoter.

The term "promoter" is used in the normal sense of the art, e.g. an RNA polymerase binding site in the Jacob-Monod theory of gene expression.

Preferably the vector comprises a tumour specific promoter enhancer.

Other preferred additional components include entities enabling efficient expression of the POI. For example, the additional component may be an enhancer. Here, the term enhancer includes a DNA sequence which binds to other protein components of the transcription initiation complex and thus facilitates the initiation of transcription directed by its associated promoter.

Preferably the vector is used to deliver the NOI and/or POI *ex vivo* and/or *in vivo* to the tumour.

The vector of the present invention is useful in gene therapy for delivering the NOI and/or the POI to a selective site.

Gene therapy includes any one or more of: the addition, the replacement, the deletion, the supplementation, the manipulation etc. of one or more nucleotide sequences in, for example,

one or more targeted sites - such as targeted cells. If the targeted sites are targeted cells, then the cells may be part of a tissue or an organ. General teachings on gene therapy may be found in Molecular Biology (Ed Robert Meyers, Pub VCH, such as pages 556-558).

By way of further example, gene therapy also provides a means by which any one or more of: a nucleotide sequence, such as a gene, can be applied to replace or supplement a defective gene; a pathogenic gene or gene product can be eliminated; a new gene can be added in order, for example, to create a more favourable phenotype; cells can be manipulated at the molecular level to treat cancer (Schmidt-Wolf and Schmidt-Wolf, 1994, Annals of Hematology 69;273-279) or other conditions - such as immune, cardiovascular, neurological, inflammatory or infectious disorders; antigens can be manipulated and/or introduced to elicit an immune response - such as genetic vaccination.

The vector of the present invention may be a viral vector or a non-viral vector. Non-viral delivery systems include but are not limted to DNA transfection methods. Here transfection includes a process using a non-viral vector to deliver a gene to a target mammalian cell. Typical transfection methods include electroporation, DNA biolistics, lipid-mediated transfection, compacted DNA-mediated transfection, liposomes, immunoliposomes, lipofectin, cationic agent-mediated, cationic facial amphiphiles (CFAs) (Nature Biotechnology 1996 14; 556), and combinations thereof. Viral delivery systems include but are not limited to adenovirus vector, an adeno-associated viral (AAV) vector, a herpes viral vector, retroviral vector, lentiviral vector, baculoviral vector. Other examples of vectors include *ex vivo* delivery systems - which include but are not limted to DNA transfection methods such as electroporation, DNA biolistics, lipid-mediated transfection, compacted DNA-mediated transfection).

Preferably the vector is a viral vector.

Preferably the vector is a retroviral vector.

In recent years, retroviruses have been proposed for use in gene therapy. Essentially, retroviruses are RNA viruses with a life cycle different to that of lytic viruses. In this

regard, when a retrovirus infects a cell, its genome is converted to a DNA form. In slightly more detail, a retrovirus is a virus which contains genomic RNA which on entry into a host cell is converted to a DNA molecule by a reverse transcriptase enzyme. The DNA copy serves as a template for the production of new RNA genomes and virally encoded proteins necessary for the assembly of infectious viral particles. Thus, a retrovirus is an infectious entity that replicates through a DNA intermediate.

There are many retroviruses and examples include: murine leukemia virus (MLV), human immunodeficiency virus (HIV), equine infectious anaemia virus (EIAV), mouse mammary tumour virus (MMTV), Rous sarcoma virus (RSV), Fujinami sarcoma virus (FuSV), Moloney murine leukemia virus (Mo-MLV), FBR murine osteosarcoma virus (FBR MSV), Moloney murine sarcoma virus (Mo-MSV), Abelson murine leukemia virus (A-MLV), Avian myelocytomatosis virus-29 (MC29), and Avian erythroblastosis virus (AEV).

A detailed list of retroviruses may be found in Coffin *et al* ("Retroviruses" 1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 758-763).

Details on the genomic structure of some retroviruses may be found in the art. By way of example, details on HIV may be found from the NCBI Genbank (i.e. Genome Accession No. AF033819).

All retroviruses contain three major coding domains, *gag, pol, env*, which code for essential virion proteins. Nevertheless, retroviruses may be broadly divided into two categories: namely, "simple" and "complex". These categories are distinguishable by the organisation of their genomes. Simple retroviruses usually carry only this elementary information. In contrast, complex retroviruses also code for additional regulatory proteins derived from multiple spliced messages.

Retroviruses may even be further divided into seven groups. Five of these groups represent retroviruses with oncogenic potential. The remaining two groups are the lentiviruses and the spumaviruses. A review of these retroviruses is presented in "Retroviruses" (1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM

Hughes, HE Varmus pp 1-25).

All oncogenic members except the human T-cell leukemia virus-bovine leukemia virus group (HTLV-BLV) are simple retroviruses. HTLV, BLV and the lentiviruses and spumaviruses are complex. Some of the best studied oncogenic retroviruses are Rous sarcoma virus (RSV), mouse mammary tumour virus (MMTV) and murine leukemia virus (MLV) and the human T-cell leukemia virus (HTLV).

The lentivirus group can be split even further into "primate" and "non-primate". Examples of primate lentiviruses include the human immunodeficiency virus (HIV), the causative agent of human auto-immunodeficiency syndrome (AIDS), and the simian immunodeficiency virus (SIV). The non-primate lentiviral group includes the prototype "slow virus" visna/maedi virus (VMV), as well as the related caprine arthritis-encephalitis virus (CAEV), equine infectious anaemia virus (EIAV) and the more recently described feline immunodeficiencey virus (FIV) and bovine immunodeficiencey virus (BIV).

A distinction between the lentivirus family and other types of retroviruses is that lentiviruses have the capability to infect both dividing and non-dividing cells (Lewis *et al* 1992 EMBO. J 11; 3053-3058, Lewis and Emerman 1994 J. Virol. 68: 510-516). In contrast, other retroviruses - such as MLV - are unable to infect non-dividing cells such as those that make up, for example, muscle, brain, lung and liver tissue.

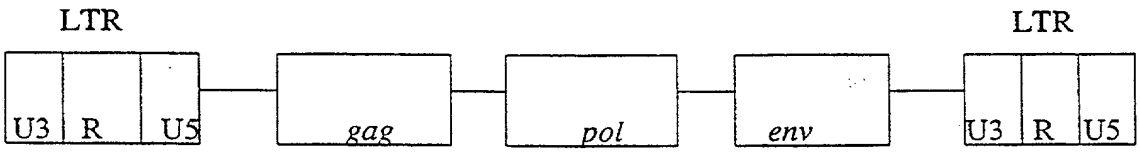
During the process of infection, a retrovirus initially attaches to a specific cell surface receptor. On entry into the susceptible host cell, the retroviral RNA genome is then copied to DNA by the virally encoded reverse transcriptase which is carried inside the parent virus. This DNA is transported to the host cell nucleus where it subsequently integrates into the host genome. At this stage, it is typically referred to as the provirus. The provirus is stable in the host chromosome during cell division and is transcribed like other cellular proteins. The provirus encodes the proteins and packaging machinery required to make more virus, which can leave the cell by a process sometimes called "budding".

As already indicated, each retroviral genome comprises genes called *gag, pol* and *env*

which code for virion proteins and enzymes. These genes are flanked at both ends by regions called long terminal repeats (LTRs). The LTRs are responsible for proviral integration, and transcription. They also serve as enhancer-promoter sequences. In other words, the LTRs can control the expression of the viral gene. Encapsidation of the retroviral RNAs occurs by virtue of a *psi* sequence located at the 5' end of the viral genome.

The LTRs themselves are indentical sequences that can be divided into three elements, which are called U3, R and U5. U3 is derived from the sequence unique to the 3' end of the RNA. R is derived from a sequence repeated at both ends of the RNA and U5 is derived from the sequence unique to the 5'end of the RNA. The sizes of the three elements can vary considerably among different retroviruses.

For ease of understanding, a simple, generic diagram (not to scale) of a retroviral genome showing the elementary features of the LTRs, *gag*, *pol* and *env* is presented below.



For the viral genome, the site of transcription initiation is at the boundary between U3 and R in the left hand side LTR (as shown above) and the site of poly (A) addition (termination) is at the boundary between R and U5 in the right hand side LTR (as shown above). U3 contains most of the transcriptional control elements of the provirus, which include the promoter and multiple enhancer sequences responsive to cellular and in some cases, viral transcriptional activator proteins. Some retroviruses have any one or more of the following genes that code for proteins that are involved in the regulation of gene expression: *tat*, *rev*, *tax* and *rex*.

As shown in the diagram above, the basic molecular organisation of a retroviral RNA genome is (5') R - U5 - *gag*, *pol*, *env* - U3-R (3'). In a retroviral vector genome *gag*, *pol*

and *env* are absent or not functional. The R regions at both ends of the RNA are repeated sequences. U5 and U3 represent sequences unique, respectively, to the 5' and 3' ends of the RNA genome. These three sets of end sequences go to form the long terminal repeats (LTRs) in the proviral DNA, which is the form of the genome which integrates into the genome of the infected cell. The LTRs in a wild type retrovirus consist of (5')U3 - R - U5 (3'), and thus U3 and U5 both contain sequences which are important for proviral integration. Other essential sequences required in the genome for proper functioning include a primer binding site for first strand reverse transcription, a primer binding site for second strand reverse transcription and a packaging signal.

With regard to the structural genes *gag, pol* and *env* themselves and in slightly more detail, *gag* encodes the internal structural protein of the virus. Gag is proteolytically processed into the mature proteins MA (matrix), CA (capsid), NC (nucleocapsid). The gene *pol* encodes the reverse transcriptase (RT), which contains both DNA polymerase, and associated RNase H activities and integrase (IN), which mediates replication of the genome. The gene *env* encodes the surface (SU) glycoprotein and the transmembrane (TM) protein of the virion, which form a complex that interacts specifically with cellular receptor proteins. This interaction leads ultimately to fusion of the viral membrane with the cell membrane.

The envelope protein is a viral protein which coats the viral particle and plays an essential role in permitting viral entry into a target cell. The envelope glycoprotein complex of retroviruses includes two polypeptides: an external, glycosylated hydrophilic polypeptide (SU) and a membrane-spanning protein (TM). Together, these form an oligomeric "knob" or "knobbed spike" on the surface of a virion. Both polypeptides are encoded by the *env* gene and are synthesised in the form of a polyprotein precursor that is proteolytically cleaved during its transport to the cell surface. Although uncleaved Env proteins are able to bind to the receptor, the cleavage event itself is necessary to activate the fusion potential of the protein, which is necessary for entry of the virus into the host cell. Typically, both SU and TM proteins are glycosylated at multiple sites. However, in some viruses, exemplified by MLV, TM is not glycosylated.

Although the SU and TM proteins are not always required for the assembly of enveloped virion particles as such, they do play an essential role in the entry process. In this regard, the SU domain binds to a receptor molecule - often a specific receptor molecule - on the target cell. It is believed that this binding event activates the membrane fusion-inducing potential of the TM protein after which the viral and cell membranes fuse. In some viruses, notably MLV, a cleavage event - resulting in the removal of a short portion of the cytoplasmic tail of TM - is thought to play a key role in uncovering the full fusion activity of the protein (Brody *et al* 1994 J. Virol. 68: 4620-4627, Rein *et al* 1994 J. Virol. 68: 1773-1781). This cytoplasmic "tail", distal to the membrane-spanning segment of TM remains on the internal side of the viral membrane and it varies considerably in length in different retroviruses.

Thus, the specificity of the SU/receptor interaction can define the host range and tissue tropism of a retrovirus. In some cases, this specificity may restrict the transduction potential of a recombinant retroviral vector. Here, transduction includes a process of using a viral vector to deliver a non-viral gene to a target cell. For this reason, many gene therapy experiments have used MLV. A particular MLV that has an envelope protein called 4070A is known as an amphotropic virus, and this can also infect human cells because its envelope protein "docks" with a phosphate transport protein that is conserved between man and mouse. This transporter is ubiquitous and so these viruses are capable of infecting many cell types. In some cases however, it may be beneficial, especially from a safety point of view, to specifically target restricted cells. To this end, several groups have engineered a mouse ecotropic retrovirus, which unlike its amphotropic relative normally only infects mouse cells, to specifically infect particular human cells. Replacement of a fragment of an envelope protein with an erythropoietin segement produced a recombinant retrovirus which then bound specifically to human cells that expressed the erythropoietin receptor on their surface, such as red blood cell precursors (Maulik and Patel 1997 "Molecular Biotechnology: Therapeutic Applications and Strategies" 1997. Wiley-Liss Inc. pp 45.).

In addition to *gag, pol* and *env*, the complex retroviruses also contain "accessory" genes which code for accessory or auxillary proteins. Accessory or auxillary proteins are defined

as those proteins encoded by the accessory genes in addition to those encoded by the usual replicative or structural genes, *gag, pol* and *env*. These accessory proteins are distinct from those involved in the regulation of gene expression, like those encoded by *tat, rev, tax* and *rex*. Examples of accessory genes include one or more of *vif, vpr, vpx, vpu* and *nef*. These accessory genes can be found in, for example, HIV (see, for example pages 802 and 803 of "Retroviruses" Ed. Coffin *et al* Pub. CSHL 1997). Non-essential accessory proteins may function in specialised cell types, providing functions that are at least in part duplicative of a function provided by a cellular protein. Typically, the accessory genes are located between *pol* and *env*, just downstream from *env* including the U3 region of the LTR or overlapping portions of the *env* and each other.

The complex retroviruses have evolved regulatory mechanisms that employ virally encoded transcriptional activators as well as cellular transcriptional factors. These *trans*-acting viral proteins serve as activators of RNA transcription directed by the LTRs. The transcriptional *trans*-activators of the lentiviruses are encoded by the viral *tat* genes. Tat binds to a stable, stem-loop, RNA secondary structure, referred to as TAR, one function of which is to apparently optimally position Tat to *trans*-activate transcription.

As mentioned earlier, retroviruses have been proposed as a delivery system (other wise expressed as a delivery vehicle or delivery vector) for *inter alia* the transfer of a NOI, or a plurality of NOIs, to one or more sites of interest. The transfer can occur *in vitro*, *ex vivo*, *in vivo,* or combinations thereof. When used in this fashion, the retroviruses are typically called retroviral vectors or recombinant retroviral vectors. Retroviral vectors have even been exploited to study various aspects of the retrovirus life cycle, including receptor usage, reverse transcription and RNA packaging (reviewed by Miller, 1992 Curr Top Microbiol Immunol 158:1-24).

In a typical recombinant retroviral vector for use in gene therapy, at least part of one or more of the *gag, pol* and *env* protein coding regions may be removed from the virus. This makes the retroviral vector replication-defective. The removed portions may even be replaced by a NOI in order to generate a virus capable of integrating its genome into a host genome but wherein the modified viral genome is unable to propagate itself due to a lack of

structural proteins. When integrated in the host genome, expression of the NOI occurs - resulting in, for example, a therapeutic effect. Thus, the transfer of a NOI into a site of interest is typically achieved by: integrating the NOI into the recombinant viral vector; packaging the modified viral vector into a virion coat; and allowing transduction of a site of interest - such as a targeted cell or a targeted cell population.

It is possible to propagate and isolate quantities of retroviral vectors (e.g. to prepare suitable titres of the retroviral vector) for subsequent transduction of, for example, a site of interest by using a combination of a packaging or helper cell line and a recombinant vector.

In some instances, propagation and isolation may entail isolation of the retroviral *gag, pol* and *env* genes and their separate introduction into a host cell to produce a "packaging cell line". The packaging cell line produces the proteins required for packaging retroviral DNA but it cannot bring about encapsidation due to the lack of a *psi* region. However, when a recombinant vector carrying a NOI and a *psi* region is introduced into the packaging cell line, the helper proteins can package the *psi*-positive recombinant vector to produce the recombinant virus stock. This can be used to infect cells to introduce the NOI into the genome of the cells. The recombinant virus whose genome lacks all genes required to make viral proteins can infect only once and cannot propagate. Hence, the NOI is introduced into the host cell genome without the generation of potentially harmful retrovirus. A summary of the available packaging lines is presented in "Retroviruses" (1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 449). However, this technique can be problematic in the sense that the titre levels are not always at a satisfactory level. Nevertheless, the design of retroviral packaging cell lines has evolved to address the problem of *inter alia* the spontaneous production of helper virus that was frequently encountered with early designs. As recombination is greatly facilitated by homology, reducing or eliminating homology between the genomes of the vector and the helper has reduced the problem of helper virus production.

More recently, packaging cells have been developed in which the *gag, pol* and *env* viral coding regions are carried on separate expression plasmids that are independently transfected into a packaging cell line so that three recombinant events are required for wild

type viral production. This strategy is sometimes referred to as the three plasmid transfection method (Soneoka *et al* 1995 Nucl. Acids Res. 23: 628-633).

Transient transfection can also be used to measure vector production when vectors are being developed. In this regard, transient transfection avoids the longer time required to generate stable vector-producing cell lines and is used if the vector or retroviral packaging components are toxic to cells. Components typically used to generate retroviral vectors include a plasmid encoding the Gag/Pol proteins, a plasmid encoding the Env protein and a plasmid containing a NOI. Vector production involves transient transfection of one or more of these components into cells containing the other required components. If the vector encodes toxic genes or genes that interfere with the replication of the host cell, such as inhibitors of the cell cycle or genes that induce apotosis, it may be difficult to generate stable vector-producing cell lines, but transient transfection can be used to produce the vector before the cells die. Also, cell lines have been developed using transient infection that produce vector titre levels that are comparable to the levels obtained from stable vector-producing cell lines (Pear *et al* 1993, PNAS 90:8392-8396).

In view of the toxicity of some HIV proteins - which can make it difficult to generate stable HIV-based packaging cells - HIV vectors are usually made by transient transfection of vector and helper virus. Some workers have even replaced the HIV Env protein with that of vesicular stomatis virus (VSV). Insertion of the Env protein of VSV facilitates vector concentration as HIV/VSV-G vectors with titres of 5×10^5 (10^8 after concentration) were generated by transient transfection (Naldini *et al* 1996 Science 272: 263-267). Thus, transient transfection of HIV vectors may provide a useful strategy for the generation of high titre vectors (Yee *et al* 1994 PNAS. 91: 9564-9568).

If the retroviral component includes an *env* nucleotide sequence, then all or part of that sequence can be optionally replaced with all or part of another *env* nucleotide sequence. Replacement of the *env* gene with a heterologous *env* gene is an example of a technique or strategy called pseudotyping. Pseudotyping is not a new phenomenon and examples may be found in WO-A-98/05759, WO-A-98/05754, WO-A-97/17457, WO-A-96/09400, WO-A-91/00047 and Mebatsion *et al* 1997 Cell 90, 841-847.

Pseudotyping can confer one or more advantages. For example, with the lentiviral vectors, the *env* gene product of the HIV based vectors would restrict these vectors to infecting only cells that express a protein called CD4. But if the *env* gene in these vectors has been substituted with *env* sequences from other RNA viruses, then they may have a broader infectious spectrum (Verma and Somia 1997 Nature 389:239-242). By way of example - workers have pseudotyped an HIV based vector with the glycoprotein from VSV (Verma and Somia 1997 *ibid*). Alternatively, *env* can be modified so as to affect (such as to alter) its specificity.

Thus, the term "recombinant retroviral vector" describes an entity (such as a DNA molecule) which contains sufficient retroviral sequences to allow an RNA transcript of the vector to be packaged in the presence of essential retroviral proteins into a retroviral particle capable of infecting a target cell. Infection of the target cell includes reverse transcription and integration into the target cell genome.

The term "recombinant retroviral vector" also covers a retroviral particle containing an RNA genome encoded by the DNA molecule. The retroviral vector will also contain non-viral genes which are to be delivered by the vector to the target cell. A recombinant retroviral vector is incapable of independent replication to produce infectious retroviral particles. Usually, a recombinant retroviral vector lacks functional *gag-pol* and/or *env* genes, or other genes encoding proteins essential for replication.

The term "targeted retroviral vector" means a recombinant retroviral vector whose ability to infect a cell or to be expressed in the target cell is restricted to certain cell types within the host organism. An example of targeted retroviral vectors is one with a genetically modified envelope protein which binds to cell surface molecules found only on a limited number of cell types in the host organism. Another example of a targeted retroviral vector is one which contains promoter and/or enhancer elements which permit expression of one or more retroviral transcripts in only a proportion of the cell types of the host organism.

Thus, the present invention provides a useful delivery system. The delivery system is capable of targeting an NOI and/or a POI to a tumour - i.e. capable of homing a NOI

and/or POI in on a tumour.

The vector may be used to administered directly to an entity - such as an organism or a cell thereof - such as *ex vivo* or *in vivo*. In this sense the vector may be delivered directly, for example, to a tumour site. Alternatively, the vector may be administered to an entity by way of a carrier - such as *ex vivo* or *in vivo*. An example of a carrier would be a liposome or a cell in which would be contained the vector. An example of a suitable carrier cell would be a haematpoietic cell, such as a myeloid cell. These carrier cells may incease the further specificity of the vector of the present invention.

These and other aspects of the present invention will now be elaborated on further.

The perceived potential of monoclonal antibody-based therapies for treatment of neoplastic disease has not been fully realised (reviewed in Scheinberg and Chapman 1995, In Monoclonal antibodies (ed. Birch and Lennox) Chapter 2.1; George *et al.*, 1994 Immunol. Today 15; 559-561). Consequently, monoclonal antibodies have been conjugated to radioisotopes, cytotoxic drugs or toxins in an attempt to improve efficacy. However, clinical trials with such conjugates have generally led to disappointing results. One of the principal reasons for the lack of efficacy of antibodies and antibody conjugates in the treatment of solid tumours is the poor penetration of solid tumours by immunoglobulins and other proteins such as immunotoxins of high molecular weight (eg. Juweid *et al.* 1992, Cancer Res. 52; 5144-5153; Epenetos *et al.* 1986 Cancer Res. 46; 3183-3191). Other reasons for lack of efficacy include the non-specific toxicity, immunogenicity and inappropriate pharmacokinetics of many immunotoxins and antibody-radionuclide conjugates introduced into the systemic circulation (reviewed in Scheinberg and Chapman 1995. In Monoclonal antibodies (ed. Birch and Lennox) Chapter 2.1).

In contrast to the general lack of *in vivo* efficacy, many monoclonal antibodies show pronounced ability to inhibit the growth of tumour cells in certain *in vitro* assays (reviewed in Sandlie and Michaelsen 1996 In Antibody engineering: a practical approach. Ed McCafferty *et al* Chapter 9). It is well established that binding of specific antigen by an antibody can lead to activation of a variety of effector functions mediated via the Fc portion

of the antibody heavy chain. The Fc regions of different immunoglobulin classes mediate different effector functions, including activation of complement cascades and binding to Fc receptors on various immune effector cells (Duncan *et al* 1988 Nature 332; 563 and 738). In *in vitro* assays, engagement of Fc receptors present on immune effector cells by antibody bound to tumour target cells can lead to destruction of the target cell by a variety of mechanisms collectively termed antibody dependent cellular cytotoxicity (ADCC). For example engagement of Fc-receptors for IgG, on human monocytes and macrophages, neutrophils and natural killer (NK) cells by antibodies of the IgG1 and IgG3 and to a much lesser extent IgG2 and IgG4 sub-classes, stimulates ADCC (Munn *et al* 1991 Cancer Res. 51; 1117-1123; Primus *et al.*, 1993 Cancer Res. 53; 3355-3361). However, the relatively poor ability of such antibodies to destroy tumours *in vivo* suggests that ADCC does not play a significant role in many of the current antibody - based therapies (George *et al*, 1994 Immunol. Today 15; 559-561). There are several possible reasons for this, including the poor penetration of antibodies into solid tumours (Yuan *et al.* 1995 Cancer Res. 55; 3752-3756) and the fact that the majority of the high-affinity receptor (FcgRI) molecules present on macrophages are normally occupied by serum IgG which will be poorly competed by specific antibody (Munn *et al* 1991 Cancer Res. 51; 1117-1123).

It has previously been shown that tumour cells transduced with genes encoding monoclonal antibodies can participate in ADCC reactions mediated by *xenogeneic* NK cells *in vitro* (Primus *et al.* 1993 Cancer Res. 53: 3355-3361). However, NK cells play little role in the destruction of tumour cells *in vivo*, in part because their killing functions are inhibited by the presence of self MHC Class I on autologous tumour cells (Correa and Raulet 1995 Immunity 2; 61-71).

It has also been postulated that tumour infiltrating lymphocytes (TILs) could be used as a vehicle to deliver antibody genes to a tumour to secrete anti-tumour antibodies at the tumour site (Tsang *et al* 1993 J. Immunother. 13; 143-152.) However, *ex vivo* transduction of TILs followed by autologous transplantation using marker genes has shown that isolated TILs show no specific homing mechanism which could allow them to return to tumour deposits (Economou *et al* 1996 J. Clin. Invest. 97; 515-521) and so any such approach is of limited value. The present invention is in contrast to these findings since

there is provided a vector that can target or deliver an NOI and/or a POI to a tumour mass (or site).

Transduction of a gene encoding a single-chain immunotoxin into human lymphokine – activated T-cells (LAK cells) has also been reported (Chen *et al* 1997 Nature 385, 78-80). In addition to the problems of re-introducing the LAK cells to the site of the tumour, such an approach also suffers from the potential drawbacks associated with being restricted to *ex vivo* use. These include the necessity of culturing the T-cells in high levels of a cytokine such as IL-2 to generate LAK cells with consequent problems in generating sufficient cells for therapy.

In one aspect, the present invention relates to the use of genetic vectors to deliver genes (such as therapeutic genes) encoding secreted tumour binding proteins (TBPs) to the interior of a tumour mass and identifies ways to target expression of TBPs to the interior of the tumour. Expression of the gene or genes encoding the TBP within the tumour mass then leads to local production of TBP with consequent reduction of tumor growth, survival or dissemination by a variety of mechanisms. Because the TBP is secreted, TBP produced by transduced cells can act not only on the transduced cell but on neighbouring tumour cells as well and hence achieve a bystander effect.

There are a number of cell types present within a tumor mass in addition to the cancerous cells. These can include cells of the tumour vasculature (eg endothelial cells) and immune cells which infiltrate the tumour, such as tumour-infiltrating lymphocytes (TIL) and macrophages (Normann 1985 Cancer Metastasis Re. 4:277-291; Leek *et al* 1996 Cancer Res. 56: 4625-4629). Any of these cell types can be targeted for expression of the TBP and can serve as a local factory within the tumour for production of TBP. Preferably, the cells in the tumour mass which are used to produce the TBP are the cancerous cells, endothelial cells or macrophages. Alternatively, the progenitors of monocytes or endothelial cells may be targeted, such as CD34-positive peripheral blood mononuclear cells (Asahara *et al*. 1997 Science 275: 964-967).

Preferably, the TBP comprises one or more binding domains capable of binding to one or

more TACSMs which are present on the cancerous cells. Thus the TBP, produced from one or more of the cell types within the tumour mass is secreted and is directed to the cancerous cells by its affinity for the TACSM. The TACSM may be selectively present on a restricted number of cell types. Thus the amount of TACSM present on the majority of the cancerous cells within the tumour mass is higher than on surrounding tissues. Preferably, the TACSM is detectably present only on tumour cells and a limited number of other tissue types in the individual containing the tumour. More preferably, the TACSM is essentially tumour-specific in the individual containing the tumour.

The one or more binding domains of the TBP may consist of, for example, a natural ligand for a TACSM, which natural ligand may be an adhesion molecule or a growth-factor receptor ligand (eg epidermal growth factor), or a fragment of a natural ligand which retains binding affinity for the TACSM. Alternatively, the binding domains may be derived from heavy and light chain sequences from an immunoglobulin (Ig) variable region. Such a variable region may be derived from a natural human antibody or an antibody from another species such as a rodent antibody. Alternatively the variable region may be derived from an engineered antibody such as a humanised antibody or from a phage display library from an immunised or a non-immunised animal or a mutagenised phage-display library. As a second alternative, the variable region may be derived from a single-chain variable fragment (scFv). The TBP may contain other sequences to achieve multimerisation or to act as spacers between the binding domains or which result from the insertion of restriction sites in the genes encoding the TBP, including Ig hinge sequences or novel spacers and engineered linker sequences.

The TBP may comprise, in addition to one or more immunoglobulin variable regions, all or part of an Ig heavy chain constant region and so may comprise a natural whole Ig, an engineered Ig, an engineered Ig-like molecule, a single-chain Ig or a single-chain Ig-like molecule. Alternatively, or in addition, the TBP may contain one or more domains from another protein such as a toxin.

In one aspect of the invention, there is provided a gene delivery system for targeting one or more genes encoding a TBP to a tumour, comprising a genetic vector encoding a TBP and

an *in vivo* gene-delivery system. The gene delivery system may be a non-viral gene delivery system such as DNA compacted with a DNA-compaction agent, or a liposome or immunoliposome which may contain DNA compacted with a DNA–compaction agent (such as a poly-lysine). The vector may be a plasmid DNA vector. Alternatively the vector may be a recombinant viral vector such as an adenovirus vector, an adeno–associated virus (AAV) vector, a herpes-virus vector or a retroviral vector in which case gene delivery is mediated by viral infection of a target cell. Preferably the vector is a recombinant retroviral vector, which may be a targeted retroviral vector. Preferably, the retroviral vector is resistant to human complement, for example by production in a human cell line.

Typically, the vector will contain a promoter to direct expression of the or each gene (such as a therapeutic gene) and may contain additional genetic elements for the efficient or regulated expression of TBP genes, including enhancers, translation initiation signals, internal ribosome entry sites (IRES), splicing and polyadenylation signals. The promoter and/or enhancer may be tissue-restricted in its activity. For example a tumour-specific promoter-enhancer, such as a 5T4 antigen gene promoter-enhancer or the CEA-gene promoter-enhancer may be used. Alternatively, or additionally, an element or elements for regulated expression may be present, such as a hypoxia regulated enhancer. An example of a hypoxia regulated expression element (HRE) is a binding element for the transcription factor HIF1. The enhancer elements or elements conferring regulated expression may be present in multiple copies. Preferably, expression of the or a gene (such as a therapeutic gene) is inducible by hypoxia (or low oxygen supply) such as may be found in a tumour mass. Most preferably, the promoter and/or enhancer directing expression of the gene (such as a therapeutic gene) contains both hypoxia–responsive elements and elements which give higher expression in tumour cells than in neighbouring non-tumour cells.

Additional vector components will be provided for other aspects of vector function such as vector maintenance, nuclear localisation, replication, and integration as appropriate using components which are well known in the art.

In a preferred embodiment of this aspect of the invention, a retroviral vector is provided for *in vivo* delivery of the gene or genes encoding the TBP to the tumour. Suitable

retroviral vectors are known in the art (see for example Gunzberg and Salmons 1996 In Gene Therapy ed. Lemoine and Cooper. Bios; and Cosset *et al.* 1995 J. Virol. 69; 7430-7436). In a particularly preferred embodiment, expression of the TBP may be enhanced in the hypoxic regions of the tumour by the inclusion of hypoxia regulated genetic elements in the retroviral vector. In this case, the hypoxia-regulated elements may be inserted into one or both of the retroviral LTRs in place of the LTR enhancer or in another position in the vector, by standard molecular biology techniques. The gene or genes encoding the TBP may be expressed from a promoter-enhancer which leads to enhanced expression in the tumour cells compared with neighbouring non-tumour cells or is preferably essentially tumour-specific. Examples of suitable promoters include the promoter-enhancer of the gene for 5T4 antigen, the promoter-enhancer of the MUC1 gene or the CEA gene.

In an other aspect of the invention there is provided a method of treating cancer comprising administering the TBP gene or genes in a gene delivery system of the first aspect of the invention either systemically or directly to the site of a tumour.

In an other aspect of the invention, is provided a gene delivery system for introducing one or more genes encoding a TBP into cells of the haematopoietic (preferably myeloid haematopoietic) cell lineage either *in vivo* or *ex vivo*. Preferably the haematopoietic (preferably myeloid haematopoietic) cells are of the monocyte-macrophage lineage or a precursor of such cells such as a CD34-positive stem cell. For *ex vivo* delivery, the genes can be inserted into a plasmid vector and delivered by one of a variety of DNA transfection methods including electroporation, DNA biolistics, lipid-mediated transfection or compacted DNA-mediated transfection. Alternatively a viral vector can be used to transduce haematopoietic (preferably myeloid haematopoietic) cells or CD34-positive stem cells *ex vivo,* such as an adenovirus vector, a retroviral vector or a lentiviral vector. The vector will contain a promoter to direct expression of the or each gene (such as a therapeutic gene) and may contain additional genetic elements for efficient or regulated expression including enhancers, translation initiation signals, internal ribosome entry sites (IRES), splicing and polyadenylation signals. The promoter, or an enhancer or splicing signals may be tissue-restricted and preferentially active in mononuclear phagocytes such as macrophages. The promoter and/or enhancer may contain elements for regulated

expression such as a hypoxia-regulated enhancer. An example of a hypoxia regulated expression element is HIF1 transcription factor response element. Such an element may be present in multiple copies. Examples of hypoxia-regulated promoters and enhancers include those from the enolase gene, the erythropoietin gene, and genes encoding glycolytic enzymes (Semenza *et al.*, 1994 J. Biol. Chem 269; 23757-23763) such as the PGK gene. Isolated HREs can be multimerised in order to increase the response to hypoxia. Additional vector components may be provided for other aspects of vector function such as vector maintenance, nuclear localisation, replication and integration as appropriate using components which are well known in the art.

After introduction of the vector into the cells *ex vivo*, the cells can be re-introduced into the patient directly or they can be stimulated to differentiate along the monocyte-macrophage differentiation pathway using appropriate combinations of cytokines and growth factors prior to re-introduction into the patient. CD34-positive cells are stimulated to differentiate using cytokines including IL-3, GMCSF and MCSF. Monocytes are differentiated either by culture attached to plastic or using GMCSF either alone or in combination with other cytokines including MCSF.

For introduction of genes (such as therapeutic genes) into haematopoietic (preferably myeloid haematopoietic) cells or CD34-positive stem cells *in vivo*, a suitable *in vivo* delivery system can be used to deliver the transcription units described above. The gene delivery system may be a non-viral gene delivery system such as DNA compacted with a DNA-compaction agent, or a liposome or immunoliposome which may contain DNA compacted with a DNA–compaction agent. Alternatively the vector may be a recombinant viral vector such as a targeted adenovirus vector, an adeno–associated viral (AAV) vector, a herpes-virus vector or a retroviral vector such as a lentiviral vector. Preferably the vector is a targeted recombinant retroviral vector, which is preferably resistant to human complement, for example by preparation of the vector from a human packaging cell line.

CD34-positive stem cells can also differentiate to form endothelial cells (Ashara *et al.* 1997 Science 275; 964-967). Such a route of differentiation for CD34 positive stem cells containing TBP encoding genes according to the invention is envisaged in addition to

differentiation to form monocytes and macrophages.

Additional vector components may be provided for other aspects of vector function such as vector maintenance, nuclear localisation, replication, and integration as appropriate using components which are well known in the art.

In a preferred embodiment of this aspect of the invention, a plasmid vector or a retroviral vector carrying a gene encoding a TBP under the control of a hypoxia regulated promoter or a promoter preferentially active in macrophages is introduced into autologous peripheral blood monocytes. The transfected monocytes are re-introduced into the patient where they migrate to the hypoxic regions of tumours permitting enhanced production of the TBP in the interior of the tumour mass. The macrophages are optionally treated with cytokines prior to re-injection into the patient. Alternatively or additionally the vector may include DNA sequences capable of expressing a cytokine gene such as a gene for IFNg, CSF-1 or GM-CSF in order to elicit the differentiation of the transfected cells. The cytokine gene may also be regulated by genetic elements which show enhanced activity at the site of the tumour.

In an other aspect of the invention, there is provided a method for treating cancer in a human or non-human mammal, comprising withdrawing an amount of blood from an individual suffering from cancer, preparing from the blood a cell preparation enriched in monocytes and macrophages or their stem-cell progenitors, introducing TBP genes into the cell preparation using a gene delivery system of the third aspect of the invention so as to bring about transfection or transduction of the monocytes and macrophages, or their stem-cell progenitors with the TBP genes, and re-introducing the transfected or transduced cells either systemically or directly to the site of the tumour. The cell preparations may optionally be treated with cytokines prior to reintroduction in order to elicit differentiation towards active macrophages.

In an other aspect of the invention is provided a method for treating cancer in a mammal, comprising administering to an individual a gene delivery system of the invention capable of expressing a TBP in cells derived from a haematopoietic (preferably myeloid

haematopoietic) origin.

In a further aspect of the invention there is provided a genetic vector comprising a gene (such a therapeutic gene) or genes encoding a TBP, operably linked to an expression regulatory element selectively functional in a cell type present within a tumour mass. The TBP in this aspect of the invention inhibits tumour function by binding to a TACSM having an essential role in tumour cell survival or dissemination. The TACSM in this aspect of the invention may be a cell surface molecule which has a role in tumour cell growth, migration or metastasis, and is present on cancerous cells or on another cell type within the tumour mass. Preferably the TACSM is present on cancerous cells or tumour vasculature or on macrophages and is a molecule such as a growth-factor receptor, a plasminogen activator, a metalloproteinase or the 5T4 antigen. The gene or genes encoding the TBP may be delivered to the interior of the tumour by any of the routes described in the above two aspects of the invention. Binding of the TBP to the corresponding TACSM blocks the function of the TACSM and thereby leads to inhibtion of growth, migration or metastasis of the tumour.

In a yet further aspect of the invention, a genetic vector comprising a gene (such as a therapeutic gene or genes) is delivered to the interior of the tumour wherein the gene (such as a therapeutic gene) encodes a TBP, which additionally contains one or more effector domains. The effector domain or domains may be activated on binding of the TBP to a TACSM leading to inhibition of tumour cell proliferation, survival or dissemination. The TACSM in this aspect of the invention is a cell surface molecule for which a specific TBP is available such as a tumour specific carbohydrate moiety, an oncofoetal antigen, a mucin, a growth-factor receptor or another glycoprotein. The TACSM is preferably an antigen restricted in its tissue distribution and found predominantly on the tumour cells and on the majority of cells within the tumour. Alternatively, the TACSM is present on tumour macrophages or the tumour vasculature. In some instances, the TACSM is not shed from the cell surface into the circulation to an appreciable extent. However, shedding may occur. By way of example, shedding of the 5T4 antigen into the stroma can serve to further localise the NOI and/or the POI to the tumour environment.

The effector domain of the present invention may possess enzymatic activity and may be for example a pro-drug activating enzyme, or it may be a non-enzyme domain. Examples of TBPs containing effector domains with enzyme activity include antibody - enzyme conjugates or fusions. Antibody - enzyme conjugates have been described including conjugates with alkaline phosphatase (Senter *et al.*, 1988 Proc. Natl. Acad. Sci. 85: 48424846); carboxypeptidase G2 (Bagshawe *et al.* 1988 Br. J. Cancer 58: 700703); P-lactamase (Shepherd *et al* 1991 Bioorg. Med. Chem. Left. 1:21-26); and Penicillin -V - amidase (Kerr *et al.* 1990 Cancer Immunol. Immunother. 31: 202-206. Antibody - enzyme fusions have also been described (Goshorn *et al* 1993 Cancer Res 53: 2123-2127; Wels *et al* 1992 Bio/Technology 10: 1 1 28-1132). Each of these examples can be used in this aspect of the invention. Additional or alternative enzymes which may be included in TBPenzyme fusions include human Carboxypeptidase Al or a mutant thereof (Smith *et al* 1997 J. Biol. Chem. 272: 15804-15816); cytosine deaminase (Mullen *et al.* 1994 Cancer Res. 54: 1503-1506); HSV thymidine kinase (Borrelli *et al.* 1988 Proc. Natl. Acad. Sci. 85: 7572-7576.); nitroreductase; P450-Reductase and a P450.

Preferably the pro-drug activating enzyme domain or domains are genetically fused to the C-terminus of an immunoglobulin or immunoglobulin domain such as a scfv or a single-chain antibody or Fab-fragment. In a particularly preferred embodiment of this aspect of the invention, the immunoglobulin domain or domains are human or humanised and the enzyme is a human enzymp- such as a Carboxypeptidase a P450 or P450-Reductase. The enzyme may be a mutant enzyme which converts a pro-drug more efficiently than does the native human enzyme. In accordance with the present invention, any enzyme that has utility in an ADEPT strategy can be used.

In each case, a suitable pro-drug is used in the treatment of the patient in combination with the appropriate pro-drug activating enzyme. Examples of pro-drugs include etoposide phosphate (used with alkaline phosphatase Senter *et al.*, 1988 Proc. Nat. Acad. Sci. 85: 4842-4846); 5-fluorocytosine (with Cytosine deaminase Mullen *et al.* 1994 Cancer Res. 54: 1503-1506); Doxorubicin-N-p-hydroxyphenoxyacetamide (with Penicillin-V-Amidase (Kerr *et al.* 1990 Cancer Immunol. Immunother. 31: 202-206); Para-N-bis(2chloroethyl) aminobenzoyl glutamate (with Carboxypeptidase G2); Cephalosporin nitrogen mustard

carbamates (with P-lactamase); SR4233 (with P450 Reducase); Ganciclovir (with HSV thymidine kinase, Borrelli *et al.* 1988 Proc. Natl. Acad. Sci. 85: 7572-7576) mustard pro-drugs with nitroreductase (Friedlos *et al.* 1997 J Med Chem 40: 1270-1275) and Cyclophosphamide (with P450 Chen *et al.* 1996 Cancer Res 56: 1331-1340).

Alternatively the effector domain may be a non-enzyme domain. Examples of non-enzyme effector domains include toxins such an exotoxin from a pseudomonad bacterium, all or part of a cytokine such as IL-2 or IFNγ, or effector domains from immunoglobulin heavy chains.

In a preferred embodiment of this aspect of the invention, the TBP contains an effector domain capable of activating macrophage FcgR I, II or III receptors. On binding of the TBP to antigen on the tumour cells, macrophages present within the hypoxic regions of the tumour are activated to destroy the tumour cells directly by phagocytosis or ADCC or are activated to secrete pro-inflammatory cytokines which serve to enhance the natural immunological response to the tumour. The TBP may contain an Fc region from an immunoglobulin, a mutant Fc region, a receptor-binding fragment of the Fc region or may contain another FcR – binding domain.

Preferably the TBP contains an entity, preferably an effector domain entity, that confers protein stability ex *vivo* and/or *in vivo*.

In accordance with the present invention, the TBP may include an intact Fc region from an IgG, (such as human IgG1 or IgG3), preferably from IgE (such as human IgGE), or a part thereof.

In one preferred embodiment of this aspect of the invention, the TBP is a Sab (single chain antibody) containing a human IgG1 constant region and a binding domain which recognises the 5T4 antigen.

In a particularly preferred embodiment of this aspect of the invention, the TBP is a Sab (single chain antibody) containing a human IgE constant region and a binding domain

which recognises the 5T4 antigen.

The effector domain may be encoded by a portion of a cDNA fused in-frame to the DNA encoding the tumour-binding domain. Alternatively a genomic fragment containing introns may be used such as a human IgG1 heavy chain constant region genomic fragment.

Here the term "intron" is used in its normal sense - e.g. an intervening sequence of DNA within a gene which is removed by RNA splicing and so is not present in the mature messenger RNA and does not code for protein. Introns can be conditional or alternatively spliced in different cell types.

Introduction of TBP-encoding genes into monocytes or macrophages may be combined with further treatments to elicit macrophage differentiation and activation. For example, cells maintained *ex vivo* may be treated with cytokines such as IFNγ, CSF-1 or GM-CSF prior to re-introduction into the patient. Alternatively, genes encoding these cytokines may be introduced into the monocytes/macrophages in the same or a different vector from the TBP genes *in vivo* or *ex vivo*. Consequently in a still further aspect of the invention there is provided a method of treating cancer in a mammal which comprises administering to an individual a combination of a cytokine or a cytokine-encoding gene and one or more TBP genes according to any of the previous aspects of the invention.

In accordance with the invention, standard molecular biology techniques may be used which are within the level of skill in the art. Such techniques are fully described in the literature. See for example; Sambrook *et al.* (1989) Molecular Cloning; a laboratory manual; Hames and Glover (1985 – 1997) DNA Cloning: a practical approach, Volumes I-IV (second edition). Methods for the engineering of immunoglobulin genes in particular are given in McCafferty *et al* (1996) Antibody engineering: a practical approach.

In a preferred aspect, the present invention relates to the delivery of TBP-encoding genes to the site of a tumour. This has considerable advantages for medical applications (such as therapeutic applications) in which TBPs are indicated since it circumvents a number of problems associated with delivery of proteins systemically in humans.

In contrast to the problems associated with production and delivery of proteins, the methods of the invention allow the delivery of genes to the site of the tumour, thus circumventing a number of production problems. The TBPs are thereby produced *in situ* in the autologous human cells, which serve as a local factory for the production of the gene-based medicament (such as a therapeutic). This has significant advantages in minimising systemic toxicity. The activity of the protein is maximal since the glycosylation of the protein shows a human pattern appropriate to the individual being treated.

The methods of the invention can be used in conjunction with direct injection into the site of the tumour or systemic delivery of, for example targeted vectors or engineered haematopoietic (preferably myeloid haematopoietic) cells or their progenitors. Systemic delivery may be particularly advantageous in a number of indications, particularly in the treatment of disseminated disease. In these cases the gene delivery system or engineered cells can be administered intravenously by bolus injection or by infusion in a suitable formulation. A pharmaceutically acceptable formulation may include an isotonic saline solution, a buffered saline solution or a tissue-culture medium. Additional formulatory agents may be included such as preservative or stabilising agents.

Thus, the present invention also encompasses a pharmaceutical composition for treating one or more individuals by gene therapy, wherein the composition comprises a therapeutically effective amount of the vector according to the present invention or the expressed product thereof. The pharmaceutical composition may be for human or animal usage. Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient.

The composition may optionally comprise a pharmaceutically acceptable carrier, diluent, excipient or adjuvant. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as - or in addition to - the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s), and other carrier agents that may aid or increase the

viral entry into the target site (such as for example a lipid delivery system).

Where appropriate, the pharmaceutical compositions can be administered by any one or more of: inhalation, in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intracavernosally, intravenously, intramuscularly or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

Thus, a preferred aspect of the present invention relates to a vector comprising (a) a NS coding for a TIP and (b) an NOI which encodes a POI; wherein the TIP is capable of recognising a tumour, such that in use the vector is capable of delivering the NOI and/or the POI to the tumour.

In one exemplary embodiment of the present invention TIP is IgG or IgE or a part thereof.

In another exemplary embodiment of the present invention, TIP is EGF or a part thereof.

In another exemplary embodiment of the present invention, TIP recognises a trophoblast cell surface antigen and at least one of the effector domains is a secreted co-stimulatory molecule. Further background teaching and details on this embodiment now follow.

The latter-mentioned aspect of the present invention relates to a process for the activation of lymphocytes and the use of activated lymphocytes in the treatment of cancer. It also relates to fusion proteins for the activation of lymphocytes, to nucleic acids encoding the fusion proteins and to vectors carrying the nucleic acids.

Lymphocytes require at least two distinct signals in order to respond to antigens by activation of effector functions (Bretscher and Cohn 1970 Science 169: 1042-1049; Crabtree 1989 Science 243: 355-361). The primary signal is specific for antigen. For B-lymphocytes, the B-cell antigen receptor (surface immunoglobulin) recognises three-dimensional epitopes on a variety of macromolecules. For T-lymphocytes, the T-cell receptor (TCR) recognises peptide antigens displayed on the surface of antigen-presenting cells by proteins of the major histocompatability (MHC) family (Weiss *et al.* 1986 Ann. Rev. Immunol. 4: 593-619).

Stimulation of the primary signal in isolation normally leads to apoptosis (programmed cell death) of the lymphocyte or leads to the establishment of a state of sustained unresponsiveness or anergy (Weiss *et al. supra*). In order to achieve activation of the lymphocyte, accessory signals are required which may be delivered by cytokines or by cell-surface co-stimulatory ligands present on antigen-presenting cells (APC).

There are a number of such co-stimulatory molecules now identified including adhesion molecules, LFA-3, ICAM-1, ICAM-2. Major co-stimulatory molecules present on APC are the members of the B7 family including B7-1 (CD80), B7-2 (CD86) and B7-3. These molecules are ligands of co-stimulatory receptors on lymphocytes including CD28 (WO92/00092), probably the most significant co-stimulatory receptor for resting T-cells. Different members of the B7 family of glycoproteins may deliver subtly different signals to T-cells (Nunes *et al.* 1996 J. Biol. Chem. 271: 1591-1598).

Established tumours, despite the fact that they commonly express unusual antigens on their surfaces, are poorly immunogenic. It has been postulated previously that one method for stimulating immune recognition of tumour cells would be to enhance antigen presentation and co-stimulation of lymphocytes in the context of tumour antigens. Transfection of the genes encoding B7-1 and B7-2, alone or in combination with cytokines, have been shown to enhance the development of immunity to experimental tumours in animal models (e.g. Leong *et al.* 1997 Int. J. Cancer 71: 476-482; Zitvogel *et al.* 1996 Eur. J. Immunol. 26:1335-1341; Cayeux et al. 1997 J. Immunol 158:2834-2841). However, in translating

these results into a practical treatment for human cancer, there are a number of significant problems to be overcome. A major problem in such studies is the need to deliver B7 genes *in vivo* to a large number of cells of the tumour to achieve efficacy. A second problem is that it is important to target expression of B7 to the tumour cells to avoid inappropriate immune cell activation directed against other cell types.

This aspect of the present invention solves these specific problems by delivering a gene encoding a secreted co-stimulatory molecule ("SCM") with binding affinity for a tumour antigen. In this way, a relatively small number of transfected cells within the tumour act as a local factory to produce the co-stimulatory molecule which is shed from the producer cell and binds to other cells in the tumour. The aspect of the present invention has the additional advantage that tumour cells need not be the target for transfection.

The SCM of the invention is a novel engineered fusion protein comprising a signal peptide for secretion from mammalian cells, at least one antigen-binding domain from an immunoglobulin or an immunoglobulin-like molecule and at least one further domain which acts as a co-stimulatory signal to a cell of the immune system. The use of combinations of SCMs containing different co-stimulatory domains is also envisaged. The SCMs are produced by expression of SCM-encoding genes in the autologous cells of the individual to be treated and hence any post-translational modifications added to the protein by the host cell are authentic and provide fully functional protein and appropriate pharmacokinetics.

WO-A-92/00092 describes truncated forms of B7-1, derived by placing a translation stop codon before the transmembrane domain, secreted from mammalian cells. In that particular case, a heterologous signal peptide from the Oncostatin M gene was used. WO-A92/00092 also describes fusion proteins which contain the extracellular domain of B7-1 fused to the Fc region of an immunoglobulin. Such molecules can bind to CD28 on T-cells and serve to stimulate T-cell proliferation. However such stimulation occurs only to a moderate extent unless the B7 or B7-derivative is immobilised on a solid surface.

Gerstmayer *et al.* (1997 J. Immol. 158: 4584-4590) describes a fusion of B7-2 to an scFv specific for ErbB2 followed by a myc epitope tag and polyhistidine tag which is secreted

when expressed in the yeast *Pichia pastoris*. This molecule retained binding for antigen and co-stimulated proliferation of T-cells prestimulated with PMA and IL-2. However, glycosylation of such a molecule is of the yeast type, which is likely to lead to inappropriate pharmacokinetics in humans.

In accordance with the present invention, any suitable co-stimulalatory domain(s) may be used. By way of example, co-stimulatory domains can be chosen from extracellular portions of the B7 family of cell-surface glycoproteins, including B7-1, B7-2 and B7-3 or other co-stimulatory cell surface glycoproteins such as but not limited to co-stimulatory receptor-ligand molecules including CD2/LFA-3, LFA-1/ICAM-1 and ICAM-3. Studies have demonstrated that T cell co-stimulation by monocytes is dependent on each of two receptor ligand pathways CD2/LFA-3 and LFA-1/ICAM-1 (Van Seventer *et al* 1991 Eur J Immunol 21: 1711-1718). In addition, it has been shown that ICAM-3, the third LFA-1 counterreceptor, is a co-stimulatory molecule for resting and activated T lymphocytes (Hernandez-Caselles *et al* 1993 Eur J Immunol 23: 2799-2806).

Other possible co-stimulatory molecules may include a novel glycoprotein receptor designated SLAM, has been identified which, when engaged, potentiates T-cell expansion in a CD28-independent manner and induces a Th0/Th1 cytokine production profile (Cocks *et al* 1995 Nature 376: 260-263).

CD6, a cell surface glycoprotein, has also been shown to function as a co-stimulatory and adhesion receptor on T cells. Four CD6 isoforms (CD6a, b, c, d) have been described (Kobarg *et al* 1997 Eur J Immunol 27: 2971-2980). A role for the very late antigen (VLA-4) integrin in the activation of human memory B cells has also been suggested (Silvy *et al* 1997 Eur J Immunol 27: 2757-2764). Endothelial cells also provide unique co-stimulatory signals that affect the phenotype of activated CD4+ T cells (Karmann *et al* 1996 Eur J Immunol 26: 610-617). A B3 protein, present on the surface of lipopolysaccharide-activated B cells, which can provide co-stimulation to resting T cells leading to a predominant release of interleukin (IL)-4 and IL-5 and negligible amounts of IL-2 and interferon gamma has been described (Vinay *et al* 1995 J Biol Chem 270: 23429-23436). The co-expression of a novel co-stimulatory T cell antigen (A6H) on T cells and tumour

cells has suggested a possible function related to common properties of these cells (Labuda *et al* 1995 Int Immunol 7: 1425-1432).

In one preferred embodiment of the invention, the co-stimulatory domain is a portion of B7-1 or B7-2, more preferably the complete extracellular portion of B7-1 or B7-2.

The SCM is formed by expression of a novel gene encoding a fusion protein containing the antigen-binding domain or domains and the co-stimulatory domain or domains. If the antigen-binding domain is comprised of a heavy and a light chain, the co-stimulatory domain is fused to one or other of the immunoglobulin chains, preferably to the heavy chain. If the antigen-binding domain is a scFv, the co-stimulatory domain is fused to the scFv. The domains can be placed in the order (N-terminus to C-terminus): antigen-binding domain followed by co-stimulatory domain; or co-stimulatory domain followed by antigen-binding domain. Preferably, the co-stimulatory domain is placed at the N-terminus followed by the antigen-binding domain. A signal peptide is included at the N-terminus, and may be for example the natural signal peptide of the co-stimulatory extracellular domain. The different domains may be separated by additional sequences, which may result from the inclusion of convenient restriction-enzyme cleavage sites in the novel gene to facilitate its construction, or serve as a peptide spacer between the domains, or serve as a flexible peptide linker or provide another function. Preferably the domains are separated by a flexible linker.

Two or more different genes encoding different SCMs may be used to achieve improved co-stimulation, or both co-stimulation of naïve T-cells and induction of memory responses. For example a gene encoding an SCM containing the B7-1 extracellular domain may be administered with a gene encoding an SCM containing the B7-2 extracellular domain.

Thus in one aspect of the invention, there is provided one or more genetic vectors capable of expressing in mammalian cells one or more secreted co-stimulatory molecules, each secreted co-stimulatory molecule comprising at least one antigen-binding domain and at least one domain from the extracellular portion of a cell-surface co-stimulatory molecule. The co-stimulatory domain may be obtained from a molecule expressed on the surface of an

antigen-presenting cell such as a B7 family member. Preferably the co-stimulatory domain is from B7-1, B7-2 or B7-3. Most preferably it is comprised of B7-1 amino acid residues 1 to approximately 215 of the mature B7-1 molecule (described in WO-A-96/00092) or amino acids 1 to approximately 225 of the mature cell-surface form of B7-2 (described in Gerstmeyer *et al.* 1997 J. Immunol. 158:4584-4590).

The genetic vector according to this aspect of the invention comprises at least a promoter and enhancer for expression in mammalian cells and a polyadenylation site. Suitable promoters and enhancers include the MIE promoter-enhancer from human cytomegalovirus or promoters which are expressed preferentially in cells present within the tumour. Such promoter-enhancers include those from the MUC1 gene , the CEA gene or the 5T4antigen gene. If two or more SCMs are expressed, the coding regions for these may be inserted into two separate vectors or a single vector may be used to express the two or more genes. In the latter case each gene is provided with a separate copy of the promoter, or an internal ribosome entry site (IRES) is used to separate the two coding sequences.

The present invention also covers the use of mutants, variants, homologues or fragments of the sequences disclosed herein.

The terms "variant", "homologue" or "fragment" in relation to the nucleotide sequences include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence providing the resultant nucleotide sequence codes for or is capable of coding for an entity having the same function as that presented herein, preferably being at least as biologically active as the same. In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant nucleotide sequence codes for or is capable of coding for an entity having the same function as that presented herein. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to the sequences shown herein. More preferably there is at least 95%, more preferably at least 98%, homology to the sequences shown herein.

In particular, the term "homology" as used herein may be equated with the term "identity".

Relative sequence homology (i.e. sequence identity) can be determined by commercially available computer programs that can calculate % homology between two or more sequences. A typical example of such a computer program is CLUSTAL.

The terms "variant", "homologue" or "fragment" are synonymous with allelic variations of the sequences.

The term "variant" also encompasses sequences that are complementary to sequences that are capable of hybridising to the nucleotide sequences presented herein. Preferably, the term "variant" encompasses sequences that are complementary to sequences that are capable of hybridising under stringent conditions (e.g. 65°C and 0.1xSSC {1x SSC = 0.15 M NaCl, 0.015 Na_3 citrate pH 7.0}) to the nucleotide sequence presented herein.

The present invention also covers nucleotide sequences that can hybridise to the nucleotide sequences of the present invention (including complementary sequences of those presented herein). In a preferred aspect, the present invention covers nucleotide sequences that can hybridise to the nucleotide sequence of the present invention under stringent conditions (e.g. 65°C and 0.1xSSC) to the nucleotide sequence presented herein (including complementary sequences of those presented herein).

The terms "variant", "homologue" or "fragment" in relation to the amino acid sequences include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acid from or to the sequence providing the resultant amino acid sequence has the same function as that presented herein, preferably being at least as biologically active as the same. In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant amino acid sequence has the same function as that presented herein. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to the sequences shown herein. More preferably there is at least 95%, more preferably at least 98%, homology to the sequences shown herein.

In summation, the present invention relates to a vector comprising (a) a NS coding for a

TIP and optionally (b) an NOI which encodes a POI; wherein the TIP is capable of recognising a tumour, such that in use the vector is capable of delivering the NOI and/or the POI to the tumour.

A preferred aspect of the present invention relates to a vector comprising (a) a NS coding for a TIP and (b) an NOI which encodes a POI; wherein the TIP is capable of recognising a tumour, such that in use the vector is capable of delivering the NOI and/or the POI to the tumour.

A further preferred aspect of the present invention relates to a vector comprising (a) a NS coding for a TIP and (b) an NOI which encodes a POI; wherein the TIP is capable of recognising a tumour, such that in use the vector is capable of delivering the NOI and/or the POI to the tumour; and wherein the TIP and POI are fused to each other.

This aspect of the present invention is advantageous as it allows for the production and delivery of, for example, a fusion product that comprises an effector component and a targetting component.

A further preferred aspect of the present invention relates to a vector comprising (a) a NS coding for a TIP and (b) an NOI which encodes a POI; wherein the TIP is capable of recognising a tumour, such that in use the vector is capable of delivering the NOI and/or the POI to the tumour; wherein the TIP and POI are fused to each other; and wherein the POI is capable of being secreted.

This aspect of the present invention is highly advantageous as it provides a means for the *in situ* production of a POI by, for example, a small number of cells for the subsequent delivery of at least a portion of the produced POI to at least one neighbouring cell. Thus, one need only infect a small number of cells to achieve a beneficial therapeutic effect.

Thus, alternatively expressed, the present invention provides the use of a vector according to the present invention as an *in situ* production factory of any one or more of the NS, NOI, POI and TIP.

In addition, the present invention provides the use of a vector according to the present invention when present in a cell to deliver an NOI and/or POI to a neighbouring cell.

A more preferred aspect of the present invention relates to a vector comprising (a) a NS coding for a TIP, (b) an NOI which encodes a POI, and (c) a nucleotide sequence that codes for a secretory entity; wherein the TIP is capable of recognising a tumour, such that in use the vector is capable of delivering the NOI and/or the POI to the tumour; wherein the TIP and POI are fused to each other; and wherein the POI is capable of being secreted.

The invention will now be further described by way of examples, which are meant to serve to assist one of ordinary skill in the art in carrying out the invention and are not intended in any way to limit the scope of the invention. Reference is made to the following Figures:

Figure 1a – which shows a DNA sequence encoding a 5T4 scFv, designated 5T4scFv.1. The sequence of the mature secreted protein is given.

Figure 1b – which shows the cDNA sequence encoding 5T4Sab1. The sequence begins with a *Hind*III restriction site followed by a translation initiation signal and a signal peptide.

Figure 2 shows the sequence of B7-1.5T4.1

Figure 3 shows a diagrammatic representation of two SCMs based on the B7-1 co-stimulatory domain; Figure 3a shows the SCM B7-1.5T4.1 and Figure 3b shows B7-1.5T4.2 in which the order of the co-stimulatory and tumour-binding domains are reversed. Sp = signal peptide; B7 ec = extracellular domain of B7-1; Vl = light chain variable domain of 5T4; Vh = heavy chain variable domain of 5T4.

Figure 4 shows the sequence of the extracellular domain of human B7-2, including the signal peptide sequence. The mature protein begins at amino acid 17. The B7-2 derived sequence is followed by a flexible linker gly-gly-gly-gly-ser.

EXAMPLES

Example 1 – Construction of 5T4 Sab and retroviral - vector delivery to tumour.

The cDNA encoding the murine 5T4 monoclonal antibody is cloned and sequenced by standard techniques (Antibody engineering: a practical approach ed McCafferty *et al.* 1996 OUP). The sequence of the variable region of the antibody can be used to construct a variety of immunoglobulin-like molecules including scFvs. The coding sequence of a 5T4 scFv, 5T4scFv.1, is shown in Figure 1a. In this molecule, the DNA sequence encodes the Vh from the mouse 5T4 monoclonal antibody followed by a 15 amino acid flexible linker and the Vl region of the mouse 5T4 antibody. The flexible linker encodes 3 copies of the amino-acid sequence gly-gly-gly-gly-ser and the DNA sequence similarity between the repeats has been minimised to avoid the risk of recombination between the repeats when plasmids containing them are grown in E. coli.

The DNA sequence shown in Figure 1a can also be used to construct a variety of single-chain antibodies (Sabs) by coupling scFv–encoding sequences to a sequence encoding a Fc region to form an in-frame fusion. A Sab is constructed using a series of DNA cassettes which can be independently varied to suit particular purposes.

Cassette 1 – Translation initiation signal and signal peptide

In order to achieve correct translation initiation and secretion from mammalian cells, the following sequence is used:

aagcttCCACCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAG
GTGTCCACTCC

This contains a convenient *Hind*III restriction site for cloning into expression vectors (lower case), the consensus translation initiation signal for mammalian cells (ANNATGPu) and the coding sequence for a signal peptide sequence from an immunoglobulin gene.

Cassette 2 – scFv

The sequence of the secreted portion of the 5T4scFv.1 is shown in Figure 1a. This molecule can be represented as Vh – $(gly_4\text{-}ser)_3$ linker - Vl.

5T4 scFv2 consists of the 5T4 variable region sequences connected in the order Vl – flexible linker Vh. In this case the linker encodes the 20 amino-acid peptide $(gly_4\text{-}ser)_4$. A longer linker improves assembly of the scFv when the V-region segments are in this order. (Pluckthun *et al* in Antibody Engineering: a practical approach, ed McCafferty *et al.* 1996 OUP).

Cassette 3 – Heavy chain Constant region

The sequence of a human g1 constant region genomic clone is given in Ellison *et al.* 1982 Nucl. Acids res. 10: 4071-4079. This sequence contains constant–region introns in addition to the coding sequence. This is fused in-frame to the 3'-end of one of the scFv sequences from Cassette 2. Vectors for convenient assembly of such constructs are described (Walls *et al.* 1993 Nucl. Acids Res. 21:2921-2929.

A cDNA of a 5T4 Sab, designated 5T4Sab1 is shown in Figure 1b, containing cassettes 1, 2 and 3.

For expression of a 5T4-specific scFv or Sab in human cells, the coding sequence is inserted into the vector pCIneo (Promega) under the control of a strong promoter and polyadenylation signal. The translation initiation signal and immunoglobulin leader (signal peptide) sequence from Cassette 1 at the 5'end of the coding region ensure efficient secretion of the scFv or Sab from mammalian cells.

For expression of an intact Ig, two separate translation cassettes are constructed, one for the heavy chain and one for the light chain. These are separated by an internal ribosome - entry site (IRES) from the picornavirus FMDV (Ramesh *et al.* 1996 Nucl. Acids Res. 24: 2697-2700. Alternatively, each cDNA is expressed from a separate copy of the hCMV

promoter (Ward and Bebbington 1995 In Monoclonal Antibodies ed Birch and Lennox.Wiley-Liss).

For production of retrovirus capable of expressing 5T4 antibody or immunoglobulin-like molecules with 5T4 specificity, the gene encoding a 5T4-based Sab, or a dicistronic message encoding heavy and light chains, is inserted into a retroviral vector in which retroviral genomic transcripts are produced from a strong promoter such as the hCMV-MIE promoter. A suitable plasmid is pHIT111 (Soneoka *et al.* 1995 Nucl. Acids Res.23; 628-633) and the required gene is inserted in place of the LacZ gene using standard techniques. The resulting plasmid, pHIT-5T4.1 is then transfected into the FLYRD18 or FLYA13 packaging cell lines (Cosset *et al.* 1995 J. Virol. 69; 7430-7436) and transfectants selected for resistance to G418 at 1 mg/ml. G418-resistant packaging cells produce high titres of recombinant retrovirus capable of infecting human cells. The virus preparation is then used to infect human cancer cells and can be injected into tumours *in vivo*. The 5T4 Sab is then expressed and secreted from the tumour cells.

In pHIT111, the MoMLV LTR promoter-enhancer is used for expression of the therapeutic gene in the target cell. The vector can also be modified so that the therapeutic gene is transcribed from an internal promoter-enhancer such as one which is active predominantly in the tumour cells or one which contains a hypoxia regulated element. A suitable promoter is a truncated HSV TK promoter with 3 copies of the mouse PGK HRE (Firth *et al.* 1994 Proc. Natl. Acad. Sci. 91: 6496-6500).

Example 2 – Transfection of macrophages/monocytes with an expression vector encoding TBP.

Peripheral blood mononuclear cells are isolated from human peripheral blood at laboratory scale by standard techniques procedures (Sandlie and Michaelsen 1996 In Antibody engineering: a practical approach. Ed McCafferty *et al.* Chapter 9) and at large scale by elutriation (eg Ceprate from CellPro). Adherent cells (essentially monocytes) are enriched by adherence to plastic overnight and cells can be allowed to differentiate along the macrophage differentiation pathway by culturing adherent cells for 1-3 weeks.

Monocytes and macrophages are transfected with an expression vector capable of expressing TBP in human cells. For constitutive high level expression, the TBP is expressed in a vector which utilises the hCMV-MIE promoter-enhancer, pCI (Promega). For hypoxia-induced expression, the hCMV promoter is replaced by a promoter containing at least one HRE. A suitable promoter is a truncated HSV TK promoter with 3 copies of the mouse PGK HRE (Firth *et al.* 1994 Proc. Natl. Acad. Sci. 91: 6496-6500).

A variety of transfection methods can be used to introduce vectors into monocytes and macrophages, including particle-mediated DNA delivery (biolistics), electroporation, cationic agent-mediated transfection (eg using Superfect, Qiagen). Each of these methods is carried out according to the manufacturer's instructions, taking into account the parameters to be varied to achieve optimal results as specified by the individual manufacturer. Alternatively, viral vectors may be used such as defective Adenovirus vectors (Microbix Inc or Quantum Biotechnologies Inc).

Example 3 – Assay for ADCC mediated by macrophages

Cells from primary human tumours or tumour cell lines which have been transduced with retrovirus expressing TBP are mixed with autologous or heterologous human macrophages, prepared as described in Example 2, for analysis of ADCC activity mediated by the TBP. Alternatively, macrophages engineered to produce TBP as described in Example 2 can be used to direct ADCC on non-transduced tumour cells.

The assay is carried out according to standard procedures (Sandlie and Michaelsen 1996 In Antibody engineering: a practical approach. Ed McCafferty *et al.* Chapter 9) with appropriate modifications. Briefly, the effector cells (macrophages or freshly isolated monocytes) are suspended at 3 x 10^6 cells/ml in the appropriate tissue culture medium (DMEM/Hepes, obtained from Life Technologies, containing 1% Foetal Calf Serum). 3 x 10^5 tumour target cells, labelled with ^{51}Cr are placed in each well of a round-bottomed microtitre plate in 0.1 ml culture medium. (Note the culture medium can include spent medium from the cells producing the TBP). 50 ml effector cells are added to the wells, the

plate is centrifuged at 300g for 2 min and incubated at 37°C for varying periods (eg 4 h) in a tissue culture incubator. The supernatant is then harvested by centrifugation and counted in a gamma counter. Results are expressed as percent lysis relative to total chromium release from an equivalent sample of target cells lysed with 0.1% Tween-20. The effector: target cell ratio can be varied in the assay to produce a titration curve.

For the prior stimulation of macrophage differentiation or priming, cytokines are added to the cultures. IFNg (Sigma) is added at between 100 and 5000 U/ml. CSF-1 or GM-CSF (Santa Cruz Biotechnology) can also be added at appropriate concentrations.

Example 4 – Analysis of efficacy in animal models

Human tumour-derived cell lines and tissues are cultured *in vivo* in genetically immunodeficient, "nude" mice according to well established techniques (see for example Strobel *et al.* 1997 Cancer Res. 57: 1228-1232; McLeod *et al.* 1997 Pancreas 14: 237-248). Syngeneic mouse models, in which a syngeneic tumour line is introduced into an immunocompetent mouse strain may also be used. These serve as suitable animal models for evaluating gene delivery systems of the invention. Vectors or engineered cells are administered systemically or directly into the tumour and tumour growth is monitored in treated and untreated animals. This system is used to define the effective dose range of the treatments of the invention and the most appropriate route of administration.

Example 5 - Construction of B7 – scFv Fusion proteins

The extracellular domain of B7-1 is defined by amino-acid residues 1 – 215 of the native human B7-1 protein. This sequence, together with its signal peptide-encoding sequence, is used to construct secreted fusion proteins which also contain the scFv derived from the 5T4 monoclonal antibody. The sequence of the 5T4 scFv is given in Figure 1a.

A DNA coding sequence is constructed using standard molecular biology techniques which encodes a fusion protein in which the N-terminus of the 5T4 scFv is fused after amino acid 215 of human B7-1. The sequence of this coding sequence, B7-1.5T4.1, is shown in

Figure 2. The fusion protein contains a flexible (gly-gly-gly-gly-ser) spacer between the B7-1 and 5T4 scFv sequences. The introduction of a convenient BamH1 restriction site at the end of the linker insertion (beginning at nucleotide 733) also allows for further linkers to be screened for optimal expression of bi-functional fusion protein. Figure 3 indicates the fusion protein in diagrammatic form. It is similarly possible to construct B7-1.5T4.2 (Figure 3b) in which the scFv is N-terminal and the B7 extracellular domain is C-terminal. In this case only the coding sequence of the mature B7-1 (without signal peptide) is required. A signal peptide such as an immunoglobulin leader sequence is added to the N-terminus of the scFv in this instance.

For fusion proteins which use the co-stimulatory extracellular domain of B7-2, the signal peptide and extracellular domain of B7-2 is used in place of B7-1 sequences. Figure 4 shows the coding sequence of the SCM B7-2.5T4.1co-stimulatory domain. It encodes the first 225 amino acids of human B7-2, preceded by its signal peptide, and a flexible linker (gly4-ser). The BamHI site at the end of this sequence can be used to insert the domain upstream of the 5T4scFv.1 (see Figure 3). The sequence includes the B7-2 signal peptide which can serve to allow secretion of this fusion protein in which the B7-2 domain is at the N-terminus of the fusion protein.

Each engineered cDNA is inserted into the mammalian expression vector pCI to allow expression in mammalian tissue culture cells. For this purpose, a linker sequence is added to the 5'-end of the coding sequence which introduces a convenient restriction site for insertion into the polylinker of pCI and adds the translation initiation signal CCACC immediately adjacent to the first ATG codon. Constructs in pCI are transfected into a suitable mammalian host cell line such as COS-1 to confirm secretion of the SCM. The transcription cassette from pCI or an appropriate segment of the transcription cassette is subsequently sub-cloned into the expression vector to be used as the gene delivery system for therapeutic use.

Example 6 - Transfection of macrophages/monocytes with an expression vector encoding an SCM.

Peripheral blood mononuclear cells are isolated from human peripheral blood at laboratory scale by standard techniques procedures (Sandlie and Michaelsen 1996 In Antibody engineering: a practical approach. Ed McCafferty *et al.* Chapter 9) and at large scale by elutriation (eg Ceprate from CellPro). Adherent cells (essentially monocytes) are enriched by adherence to plastic overnight and cells can be allowed to differentiate along the macrophage differentiation pathway by culturing adherent cells for 1 – 3 weeks.

Monocytes and macrophages are transfected with an expression vector capable of expressing SCM in human cells. For constitutive high level expression, the SCM is expressed in a vector which utilises the hCMV-MIE promoter-enhancer, pCI (Promega). For hypoxia-induced expression, the hCMV promoter is replaced by a promoter containing at least one HRE. A suitable promoter is a truncated HSV TK promoter with 3 copies of the mouse PGK HRE (Firth *et al.* 1994 Proc. Natl. Acad. Sci. 91: 6496-6500).

A variety of transfection methods can be used to introduce vectors into monocytes and macrophages, including particle-mediated DNA delivery (biolistics), electroporation, cationic agent-mediated transfection (eg using Superfect, Qiagen). Each of these methods is carried out according to the manufacturer's instructions, taking into account the parameters to be varied to achieve optimal results as specified by the individual manufacturer. Alternatively, viral vectors may be used such as defective Adenovirus vectors (Microbix Inc or Quantum Biotechnologies Inc).

Example 7 - Analysis of SCM binding to CTLA-4 and 5T4-antigen expressing cells

The B7-1 or B7-2 domains are expected to bind specifically to CD28 and CTLA-4 present on human T-cells. Binding to T-cells or Chinese hamster ovary cells transfected with human CTLA-4 or CD28 is determined using FACS analysis as follows. 5 x 10^5 CTLA-4 expressing target cells or equivalent cells lacking CTLA-4 (untransfected CHO cells) are incubated with 0.1 ml culture supernatant from COS-1 cells transiently transfected with

SCM genes for 1 h at 4oC. The cells are washed and incubate with 1 mg monoclonal antibody specific for the B7 domain (eg Mab 9E10) followed by FITC-labelled goat anti-mouse IgG (Pharmingen) and analysis by FACS.

Binding of scFv to 5T4-antigen is similarly assessed using target cells expressing 5T4-antigen (5T4-transfected A9 cells) or control cells (A9).

Example 8 - Analysis of co-stimulatory activity

An established mouse cell line of Balb/c origin such as HC11 cells is transfected with the cDNA encoding human 5T4-antigen (Myers *et al.* 1994 J. Biol. Chem. 269; 9319-9324) inserted in the expression vector pCIneo.

Splenic T-cells from Balb/c mice are isolated by standard procedures (Johnstone and Thorpe 1996 In Immunochemistry in Practice. Blackwell. Chapter 4). T-cells are pre-stimulated by incubation for 1 – 2 days in medium containing 10ng/ml PMA (Sigma) and 100 U/ml human IL-2 (Boehringer Mannheim). HC11-5T4 cells are incubated at 10^4 cells /well of a 96-well tissue culture tray for 2 h with up to 0.1ml supernatant from COS cells transfected with SCM gene. Up to 10^5 pre-stimulated T-cells are added to each well, the cells are pulsed with 0.25 mCi / well ^{3}H-thymidine and incorporation of ^{3}H-thymidine is measured using a liquid scintillation counter after 24h.

Incorporation of ^{3}H-thymidine is anticipated to be enhanced by the presence of SCM.

Example 9 - Analysis of co-stimulation in animal models.

HC11 cells transfected with the human 5T4-antigen gene (Example 4) are grown as tumours in Balb/c mice. SCM genes B7-1.5T4.1 or B7-2.5T4.1 or a combination of both genes are introduced into the tumour cells prior to implantation and the growth of the tumours and the growth of control tumours which do not express SCM genes *in vivo* are monitored.

It is believed that the expression of SCM genes lead to significant reduction in tumour growth.

Example 10 - Construction of a B7-1/ScFv, specific for human 5T4, fusion protein

Standard molecular biology techniques are used to construct a fusion protein consisting of the leader sequence and extracellular domain of B7-1, fused via a flexible linker to the V_H and V_L of the murine Mab 5T4 specific to human 5T4.

The flexible linker, used to join the extracellular domain of B7.1 and the ScFv, was constructed by annealing two homologous oligonucleotides with engineered 5' Sma I and 3' Spe I sites - using oligonucleotides

upper

5' GGG GGT GGT GGG AGC GGT GGT GGC GGC AGT GGC GGC GGC GGA A 3'

and lower

5' CTA GTT CCG CCG CCG CCA CTG CCG CCA CCA CCG CTC CCA CCA CCC CC 3'

The linker is cloned into pBluescript (Stratagene) via Sma I and Spe I to produce pLINK. The signal peptide (sp) and extracellular domain of murine B7.1 were ampified by PCR from pLK444-mB7.1 (supplied by R. Germain NIH, USA) via primers that introduce 5' EcoRI and 3' Sma I sites - primers forward

5' C TCG AAT TCC ACC ATG GCT TGC AAT TGT CAG TTG ATG C 3'

reverse

5' CTC CCC GGG CTT GCT ATC AGG AGG GTC TTC 3'

The B7.1 PCR product was cloned into pLINK via Eco RI and Sma I to form pBS/B7Link.

The V_H and V_L of the 5T4 specific ScFv was amplified via primers -

forward primer

5' CTC ACT AGT GAG GTC CAG CTT CAG CAG TC 3'

reverse primer

5' CTC GCG GCC GCT TAC CGT TTG ATT TCC AGC TTG GTG CCT CCA CC 3'

that introduce 5' Spe I and 3' Not I sites from pHEN1-5T4 ScFv. PBS/B7Link was digested with Spe I and Not I and ligated with the ScFv to form OBM 233 consisting of the sequence shown as SEQ ID No. 5: B7 Link scFv sequence

This fusion can be used to construct a recombinant vector e.g. retrovirus, Lentivirus, adenovirus, poxvirus, vaccinia virus, baculovirus. Such vectors can be used to inject patient tumours directly. To deliver the fusion protein to tumour cells the recombinant vector is used to transduce macrophages/monocytes/CD34+ cells *ex vivo* before injection back into patients. These cells will traffic to tumours. The ScFv will bind to a specific tumour antigen expressed on the surface of tumour cells e.g. 5T4 (Myers et al 1994 JBC). B7 is found on the surface of professional antigen presenting cells e.g. macrophages, dendritic cells and B cells. It interacts with it ligands CD28 and CTL-A4 located on CD4 and CD8 cells. The simultaneous interaction of B7-CD28/CTL-A4 and MHC-peptide/T cell receptor leads to a pronounced increase in IL-2 which promotes CD8 (cytotoxic T cell) expansion (Linsley PS, Brady W, Grosmaire L, Aruffo A, Damle NK, Ledbetter JA J Exp Med 1991 Mar 1;173(3):721-730 Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and Il-2 mRNA accumulation.) Tumour cells that have been B7 tranfected with B7 have been shown retardation in animal models (Townsend SE, Allison JP Science 1993 15;259(5093):368-370).

Example 11 - Transient expression and purification of B7-1/ScFv and LScFv

For transient expression of B7-1/ScFv the human CMV expression plasmid pCIneo (Promega) was used. B7/ScFv was excised from OBM 233 by digestion with EcoR I/Not I and cloned into pCIneo that was previously digested with EcoRI/Not I. Transient expression of recombinant protein is made by transfection of 293T cells with the relevant plasmid using calcium phosphate (Profectin, Promega). Conditions used were similar to those recommended by the manufacturer. To reduce bovine serum contamination serum free optimem media (Gibco BRL). After 36-48 hours transfection supernatants were harvested and spun through a Centriprep (Amicon, Glos. UK) 10 filter (all proteins larger than 10 kDa are purified/concentrated) and a Centricon (Amicon) 10 filter. Supernatants are concentrated approximately 30 fold.

For B7-1 to be biologically functional it must be able to display binding with one of it's natural ligands either CTLA-4 or CD28 found on the surface of specific populations of T cells (e.g CD4+). The biological activity B7-1/ScFv fusion protein was analysed for simultaneous interaction with its natural ligand CTLA-4 (in the form of CTLA4-Ig supplied by Ancell, MN, USA) and A9 cells expressing human 5T4. Briefly: approximately $5x10^5$ A9-h5T4 cells were incubated with 100ul of either B7.1/ScFv or LScFv supernatant in a U bottom 96 well plate at 4°C for 1 hour. After washing cells were incubated with CTLA4-Ig (Ancell) for 1 hour. After washing, bound CTLA4-Ig was detected using an FITC conjugated anti-mouse Ig (Dako).

Results show obvious binding of CTLA-Ig with the B7-1 extracellular domain, bound via the ScFv, to the surface of human 5T4 positive A9 cells. The lack of binding activity with 5T4 negative A9 cells further illustrates that the interaction of B7 with CTLA4-Ig and ScFv with 5T4 are specific.

Example 12 - ScFv-IgG fusion example

Construction of ScFv-IgG

The sequence encoding a translation initiation sequence and the human immunoglobulin kappa light chain signal peptide is synthesised as two complementary single stranded oligonucleotides which when annealed also contain an internal *Xho I* site at the 5' end and in addition leave a *Xba I* compatible 5' overhang and a *Pst I* compatible 3' overhang

ctagactcgagCCACC ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC GAG GTC CAG ctgca

and

g CTG GAC CTC GGA GTG GAC ACC TGT AGC TGT TGC TAC CAA GAA GAG GAT GAT ACA GCT CCA TCC CAT GGTGGctcgagt

This is then cloned into pBluescript II (Stratagene) restricted with *Xba I* and *Pst I* to create pBSII/Leader.

The 5T4 scFv is amplified by PCR from pHEN1 using oligonucleotides which incorperate a *Pst I* site at the 5' end of the product and a *Hind III* at the 3' end

GTC CAG CTG CAG CAG TCT GG

and

CG TTT GAT TTC AAG CTT GGT GC

This is then restricted with those enzymes and inserted into pBSII/Leader restricted with the same enzymes, creating pBSII/Leader/scFv.

The HIgG 1 constant region is amplified by PCR from the cloned gene using oligonucleotides which incorperate a *Hind III* site at the 5' end and a *Xho I* site at the 3' end

gcgc AAG CTT gaa atc aaa cgg GCC TCC ACC AAG GGC CCA

and

gcgc ctcgag TCA TTT ACC CGG AGA CAG GG

This is then restricted with those enzymes and inserted into pBSII/Leader/scFv restricted with the same enzymes, creating pBSII/Leader/scFv/HG1. The sequence for this construct is shown in the Figures.

This fusion can be used to construct a recombinant vector e.g. retrovirus, Lentivirus, adenovirus, poxvirus, vaccinia virus, baculovirus. Such vectors can be used to inject patient tumours directly. To deliver the fusion protein to tumour cells the recombinant vector is used to transduce macrophages/monocytes/CD34+ cells *ex vivo* before injection back into patients. These cells will traffic to tumours. The ScFv will bind to a specific tumour antigen expressed on the surface of tumour cells e.g. 5T4 (Myers et al 1994 JBC). Bound IgG will promote specific tumour destruction via a collection of mechanisms collectively known as antibody dependent cellular cytotoxicity (Munn et al Can res 1991 *ibid*, Primus et al 1993 Cancer Res *ibid*).

Example 13 - Construction of ScFv-IgE1 (human IgE1 heavy constant region)

A similar fusion construct of 5T4 scFv – human IgE constant heavy chain is made consisting of the sequence shown as SEQ ID No. 6.

This fusion construct is made by amplifying the human IgE1 constant heavy region by PCR cDNA derived from human B-cells RNA by RT and subsequently using oligonucleotides which incorporate a *Hind III* site at the 5' end and a *Xho I* site at the 3' end

gcgc AAG CTT gaa atc aaa cgg GCC TCC ACA CAG AGC CCA

and

gcgc ctcgag TCA TTT ACC GGG ATT TAC AGA

This is then restricted with those enzymes and inserted into pBSII/Leader/scFv restricted with the same enzymes, creating pBSII/Leader/scFv/HE1.

As described above the ScFv-IgE construct can be incorporated into a recombinant viral vector for use in gene therapy of cancer e.g. inject patient tissue directly or to transduce patient derived macrophages/moncytes/CD34+ cells *ex vivo*. The fusion protein will be secreted and will bind to tumour cells bearing the antigen that the ScFv is specific for. Binding of IgE to tumour cells should promote a strong histamine response via activation of mast cells. This will lead to a strong inflammatory response and destruction tumour cells as is reported for IgE cytotoxic destruction of parasites e.g. helminth larvae (Capron M 1988 Eosinophils in diseases: receptors and mediators. In progress in allergy and clinical immunology (Proc. 13th Int. Congress of Allergy and Clinical Immunology) Hogrefe & Huber Toronto p6). Such inflammation and tumour destruction should initiate the recruitment of other immune effector cells. Past reports indicate that treatment with an MMTV antigen specific IgE Mab leads to protection from a tumour expressing MMTV antigen (Nagy E Istanvan B, Sehon AH 1991 Cancer Immunol. Immunotherapy vol 34:63-69).

Example 14 - Construction of B7/EGF

B7 – EGF Synthetic Gene.

A fusion construct of B7 – EGF is made by inserting a PCR product amplified from the region of the gene encoding the mature EGF peptide (see accession number X04571) into pBS/B7 Link. This construct has the sequence shown as SEQ ID No. 7.

Using cDNA derived by RT of RNA isolated from a cell line such the 293 human kidney line (ATCC: CRL1573), the DNA is amplified by PCR using oligonucleotides containing a *Spe I* restriction enzyme site at the N-terminus and a stop codon and a *Not I* site at the C-terminus

GG ACT AGT AAT AGT GAC TCT GAA TGT CCC

and

ATT AGC GGC CGC TTA GCG CAG TTC CCA CCA CTT C

The resulting product is digested with those enzymes and ligated to pBS/B7 Link which has been restricted with the same enzymes creating pBS/B7 Link EGF. The B7 Link EGF cassette is then excised with *Eco RI* and *Not I* and inserted into a derivative of pHIT111 (Soneoka *et al*, 1995, Nucl Acid Res 23; 628) which no longer carries the *LacZ* gene

An alternative to using ScfV is to use growth factors that have a high affinity to their corresponding receptor e.g. epidermal growth factor which binds to several receptors including erb-2 which is highly associated with tumourgenesis.

As described above the fusion construct can be incorporated into a recombinant viral vector for use in gene therapy e.g. inject patient tissue directly or to transduce patient derived macrophages/moncytes/CD34+ cells ex vivo. The fusion protein will be secreted and will bind to tumour cells bearing the erb-2 antigen.

Epidermal growth factor (EGF) will bind to its ligand erb-2 (an EGF receptor) thus obviating the requirement of a ScFv. Erb-2 is highly associated with tumour cells (Hynes NE Semin Cancer Biol 1993 Feb;4(1):19-26, Amplification and over expression of the erbB-2 gene in human tumors: its involvement in tumor development, significance as a prognostic factor, and potential as a target for cancer therapy). B7 is found on the surface of professional antigen presenting cells e.g. macrophages, dendritic cells and B cells. It

interacts with it ligands CD28 and CTL-A4 located on CD4 and CD8 cells. The simultaneous interaction of B7-CD28/CTL-A4 and MHC-peptide/T cell receptor leads to massive increase in IL-2 which promotes CD8 (cytotoxic T cell) expansion (Linsley PS, Brady W, Grosmaire L, Aruffo A, Damle NK, Ledbetter JA J Exp Med 1991 Mar 1;173(3):721-730 Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation.) Tumour cells that have been B7 transfected with B7 have shown retardation in animal models (Townsend SE, Allison JP Science 1993 15;259(5093):368-370 Tumor rejection after direct costimulation of CD8+ T cells by B7-transfected melanoma cells). It is has been reported that B7 will enhance the CTL response to tumour antigens specific to tumour cells thus leading to the destruction of all such cells.

Example 15 - Production of cell lines expressing fusion constructs

The ScFv-IgG gene was excised from pBSII/L/ScFv/hIgG1 by Xho I digestion, and cloned into pLXSN via the Xho I site, to make pLXSN/ScFv-IgG, such that after chromosomal integration it is under transcriptional control of the LTR. Virus was made in the human kidney cell line 293T by co-transfecting plasmids containing the MLV gap-pol genes (pCIEGPPD) and and the VSV G envelope (pRV67) using the triple plasmid HIT system (Landau & Littman 1992 J Virol 66 5110, Soneoka Y et al 1995 NAR 23:628-633). Virus is harvested after 48 hours and used to transduce BHK-21 cells (ATCC# CCL-10). Approximately 24 hours post-transduction, transduced cells are selected by the addition of 1mg/ml G418 (Gibco BRL) to culture medium. The supernatant from positive colonies was harvested and concentrated by centrifugation through a Centriprep (Amicon, Glos. UK) 10 filter (all proteins larger than 10 kDa are purified/concentrated) and a Centricon (Amicon) 10 filter. Supernatants were concentrated approximately 30 fold.

Other fusion proteins are cloned into pLXSN via the Xho I site and expressed and concentrated using a similar protocol.

FACS analysis of fusion protein binding with cells expressing specific ligand

To determine if the ScFv-IgG fusion protein is specific for its antigen, human 5T4, FACS analysis of a human bladder carcinoma tumour line (EJ) or a stable murine cell line expressing h5T4, A9-h5T4 (Myers et al 1994 JBC) and a 5T4 negative line A9-neo was carried out. Approximately $5x10^5$ A9 or EJ cells, in a round bottom 96 well plate (Falcon) were incubated with 100ul of a 1:5 dilution of concentrated supernatant (as described above) for 1 hour at 4oC. After washing, bound protein is detected using an anti human IgG/FITC conjugated antibody (Dako). Cells were analysed on a Becton Dickinson FACS machine. FACS results show that there is at least a 1 log shift in fluorescence activity in those 5T4 positive cells treated with the ScFv-IgG construct compared to the negative control construct consisting of the ScFv protein alone. A9 neo FACS shows that there is no non-specific binding of the ScFv component of the fusion protein.

FACS analysis of ScFv-IgE is carried out similar to above except that anti-human IgE-FITC (Dako) is used to detect binding of the fusion protein.

The B7/EGF fusion protein is analysed for binding using FACS and HC11-erb-2 positive cells (Hynes et al 1990). CTLA4-Ig (Ancell, USA) is used to analyse the bioactivity of the B7 component of the bound fusion protein. Anti-mouse IgG-FITC is used to show CTLA-4 binding.

Summary

The present invention therefore provides a means for delivering, for example, therapeutic compounds to a tumour site.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTINGS

SEQ ID NO. 1

See Figure 1a.

SEQ ID NO.2

See Figure 1b.

SEQ ID NO. 3

See Figure 2

SEQ ID NO.4

See Figure 4

SEQ ID NO. 5

ATGGCTTGCAATTGTCAGTTGATGCAGGATACACCACTCCTCAAGTTTCCATGTCCAAGGCTCATTCTTCTCTTTGTGCT
80GCTGATTCGTCTTTCACAAGTGTCTTCAGATGTTGATGAACAACTGTCCAAGTCAGTGAAAGATAAGGTATTGCTGCCTT
160GCCGTTACAACTCTCCGCATGAAGATGAGTCTGAAGACCGAATCTACTGGCAAAAACATGACAAAGTGGTGCTGTCTGTC
240ATTGCTGGGAAACTAAAAGTGTGGCCCGAGTATAAGAACCGGACTTTATATGACAACACTACCTACTCTCTTATCATCCT
320GGGCCTGGTCCTTTCAGACCGGGGCACATACAGCTGTGTCGTTCAAAAGAAGGAAAGAGGAACGTATGAAGTTAAACACT
400TGGCTTTAGTAAAGTTGTCCATCAAAGCTGACTTCTCTACCCCCAACATAACTGAGTCTGGAAACCCATCTGCAGACACT
480AAAAGGATTACCTGCTTTGCTTCCGGGGGTTTCCCAAAGCCTCGCTTCTCTTGGTTGGAAAATGGAAGAGAATTACCTGG
560CATCAATACGACAATTTCCCAGGATCCTGAATCTGAATTGTACACCATTAGTAGCCAACTAGATTTCAATACGACTCGCA
640ACCACACCATTAAGTGTCTCATTAAATATGGAGATGCTCACGTGTCAGAGGACTTCACCTGGGAAAAACCCCCAGAAGAC
720CCTCCTGATAGCAAGCCCGGGGGTGGTGGGAGCGGTGGTGGCGGCAGTGGCGGCGGCGGAACTAGTGAGGTCCAGCTTCA
800GCAGTCTGGACCTGACCTGGTGAAGCCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGTTACTCATTCACTGGCT
880ACTACATGCACTGGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTGGACGTATTAATCCTAACAATGGTGTTACT
960CTCTACAACCAGAAATTCAAGGACAAGGCCATATTAACTGTAGACAAGTCATCCACCACAGCCTACATGGAGCTCCGCAG
1040CCTGACATCTGAGGACTCTGCGGTCTATTACTGTGCAAGATCTACTATGATTACGAACTATGTTATGGACTACTGGGGTC

1120AAGTAACTTCAGTCACCGTCTCTTCAGGTGGTGGTGGGAGCGGTGGTGGCGGCACTGGCGGCGGCGGATCTAGTATTGTG
1200ATGACCCAGACTCCCACATTCCTGCTTGTTTCAGCAGGAGACAGGGTTACCATAACCTGCAAGGCCAGTCAGAGTGTGAG
1280TAATGATGTAGCTTGGTACCAACAGAAGCCAGGGCAGTCTCCTACACTGCTCATATCCTATACATCCAGTCGCTACGCTG
1360GAGTCCCTGATCGCTTCATTGGCAGTGGATATGGGACGGATTTCACTTTCACCATCAGCACTTTGCAGGCTGAAGACCTG
1440GCAGTTTATTTCTGTCAGCAAGATTATAATTCTCCTCCGACGTTCGGTGGAGGCACCAAGCTGGAAATCAAACGGTAA 1518

SEQ ID NO.6

CTCGAGCCACCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCGAGGTCCAGCTG
80CAGCAGTCTGGACCTGACCTGGTGAAGCCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGTTACTCATTCACTGG
160CTACTACATGCACTGGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTGGACGTATTAATCCTAACAATGGTGTTA
240CTCTCTACAACCAGAAATTCAAGGACAAGGCCATATTAACTGTAGACAAGTCATCCACCACAGCCTACATGGAGCTCCGC
320AGCCTGACATCTGAGGACTCTGCGGTCTATTACTGTGCAAGATCTACTATGATTACGAACTATGTTATGGACTACTGGGG
400TCAAGTAACTTCAGTCACCGTCTCTTCAGGTGGTGGTGGGAGCGGTGGTGGCGGCACTGGCGGCGGCGGATCTAGTATTG
480TGATGACCCAGACTCCCACATTCCTGCTTGTTTCAGCAGGAGACAGGGTTACCATAACCTGCAAGGCCAGTCAGAGTGTG
560AGTAATGATGTAGCTTGGTACCAACAGAAGCCAGGGCAGTCTCCTACACTGCTCATATCCTATACATCCAGTCGCTACGC
640TGGAGTCCCTGATCGCTTCATTGGCAGTGGATATGGGACGGATTTCACTTTCACCATCAGCACTTTGCAGGCTGAAGACC
720TGGCAGTTTATTTCTGTCAGCAAGATTATAATTCTCCTCCGACGTTCGGTGGAGGCACCAAGCTTGAAATCAAACGGGCC
800TCCACACAGAGCCCATCCGTCTTCCCCTTGACCCGCTGCTGCAAAAACATTCCCTCCAATGCCACCTCCGTGACTCTGGG
880CTGCCTGGCCACGGGCTACTTCCCGGAGCCGGTGATGGTGACCTGGGACACAGGCTCCCTCAACGGGACAACTATGACCT
960TACCAGCCACCACCCTCACGCTCTCTGGTCACTATGCCACCATCAGCTTGCTGACCGTCTCGGGTGCGTGGGCCAAGCAG
1040ATGTTCACCTGCCGTGTGGCACACACTCCATCGTCCACAGACTGGGTCGACAACAAAACCTTCAGCGTCTGCTCCAGGGA
1120CTTCACCCCGCCCACCGTGAAGATCTTACAGTCGTCCTGCGACGGCGGCGGGCACTTCCCCCCGACCATCCAGCTCCTGT
1200GCCTCGTCTCTGGGTACACCCCAGGGACTATCAACATCACCTGGCTGGAGGACGGGCAGGTCATGGACGTGGACTTGTCC
1280ACCGCCTCTACCACGCAGGAGGGTGAGCTGGCCTCCACACAAAGCGAGCTCACCCTCAGCCAGAAGCACTGGCTGTCAGA
1360CCGCACCTACACCTGCCAGGTCACCTATCAAGGTCACACCTTTGAGGACAGCACCAAGAAGTGTGCAGATTCCAACCCGA
1440GAGGGGTGAGCGCCTACCTAAGCCGGCCCAGCCCGTTCGACCTGTTCATCCGCAAGTCGCCCACGATCACCTGTCTGGTG
1520GTGGACCTGGCACCCAGCAAGGGGACCGTGAACCTGACCTGGTCCCGGGCCAGTGGGAAGCCTGTGAACCACTCCACCAG
1600AAAGGAGGAGAAGCAGCGCAATGGCACGTTAACCGTCACGTCCACCCTGCCGGTGGGCACCCGAGACTGGATCGAGGGGG
1680AGACCTACCAGTGCAGGGTGACCCACCCCCACCTGCCCAGGGCCCTCATGCGGTCCACGACCAAGACCAGCGGCCCGCGT
1760GCTGCCCCGGAAGTCTATGCGTTTGCGACGCCGGAGTGGCCGGGGAGCCGGGACAAGCGCACCCTCGCCTGCCTGATCCA
1840GAACTTCATGCCTGAGGACATCTCGGTGCAGTGGCTGCACAACGAGGTGCAGCTCCCGGACGCCCGGCACAGCACGACGC
1920AGCCCCGCAAGACCAAGGGCTCCGGCTTCTTCGTCTTCAGCCGCCTGGAGGTGACCAGGGCCGAATGGGAGCAGAAAGAT
2000GAGTTCATCTGCCGTGCAGTCCATGAGGCAGCGAGCCCCTCACAGACCGTCCAGCGAGCGGTGTCTGTAAATCCCGGTAA
2080ATGAGAGCTC 2090

SEQ ID NO. 7

ATGGCTTGCAATTGTCAGTTGATGCAGGATACACCACTCCTCAAGTTTCCATGTCCAAGGCTCATTCTTCTCTTTGTGCT
80GCTGATTCGTCTTTCACAAGTGTCTTCAGATGTTGATGAACAACTGTCCAAGTCAGTGAAAGATAAGGTATTGCTGCCTT
160GCCGTTACAACTCTCCGCATGAAGATGAGTCTGAAGACCGAATCTACTGGCAAAAACATGACAAAGTGGTGCTGTCTGTC
240ATTGCTGGGAAACTAAAAGTGTGGCCCGAGTATAAGAACCGGACTTTATATGACAACACTACCTACTCTCTTATCATCCT
320GGGCCTGGTCCTTTCAGACCGGGGCACATACAGCTGTGTCGTTCAAAAGAAGGAAAGAGGAACGTATGAAGTTAAACACT
400TGGCTTTAGTAAAGTTGTCCATCAAAGCTGACTTCTCTACCCCCAACATAACTGAGTCTGGAAACCCATCTGCAGACACT
480AAAAGGATTACCTGCTTTGCTTCCGGGGGTTTCCCAAAGCCTCGCTTCTCTTGGTTGGAAAATGGAAGAGAATTACCTGG
560CATCAATACGACAATTTCCCAGGATCCTGAATCTGAATTGTACACCATTAGTAGCCAACTAGATTTCAATACGACTCGCA
640ACCACACCATTAAGTGTCTCATTAAATATGGAGATGCTCACGTGTCAGAGGACTTCACCTGGGAAAAACCCCCAGAAGAC
720CCTCCTGATAGCAAGCCCGGGGGTGGTGGGAGCGGTGGTGGCGGCAGTGGCGGCGGCGGAACTAGTAATAGTGACTCTGA
800ATGTCCCCTGTCCCACGATGGGTACTGCCTCCATGATGGTGTGTGCATGTATATTGAAGCATTGGACAAGTATGCATGCA
880ACTGTGTTGTTGGCTACATCGGGGAGCGATGTCAGTACCGAGACCTGAAGTGGTGGGAACTGCGC 945

CLAIMS

1. A vector comprising a nucleotide sequence ("NS") coding for a tumour interacting protein ("TIP") and optionally comprising a nucleotide sequence of interest ("NOI") which NOI encodes a product of interest ("POI"); wherein the TIP is capable of recognising a tumour, such that in use the vector is capable of delivering the NOI and/or the POI to the tumour.

2. A vector according to claim 1 wherein the vector comprises the NOI.

3. A vector according to claim 2 wherein the NOI is a therapeutic NOI and/or the POI is a therapeutic POI.

4. A vector according to any one of the preceding claims wherein in use the vector is capable of delivering the NOI and/or the POI to the interior of a tumour mass.

5. A vector according to any one of the preceding claims wherein the TIP is or comprises a tumour binding protein ("TBP").

6. A vector according to any one of the preceding claims wherein the TIP is a TBP.

7. A vector according to any one of the preceding claims wherein the NS and/or the TIP comprises at least one tumour binding domain capable of interacting with at least one tumour associated cell surface molecule ("TACSM").

8. A vector according to claim 7 wherein the TACSM is selectively expressed on one cell type or on a restrictive number of cell types.

9. A vector according to any one of the preceding claims wherein in use the vector is capable of delivering the NOI and/or the POI to a selective tumour site.

10. A vector according to any one of the preceding claims wherein the TIP is or comprises at least part of an antibody.

11. A vector according to any one of the preceding claims wherein the TIP recognises a tropoblast cell surface antigen.

12. A vector according to claim 11 wherein the TIP recognises the 5T4 antigen.

13. A vector according to any one of the preceding claims wherein the NS and NOI and/or the TIP and POI are linked together.

14. A vector according to claim 13 wherein the TIP and POI are directly linked together.

15. A vector according to any one of the preceding claims wherein any one or more of the NS, NOI, TIP and the POI further comprise at least one additional functional component.

16. A vector according to any one of the preceding claims wherein at least the TIP and/or POI further comprise at least one additional functional component.

17. A vector according to claim 15 or16 wherein the additional functional component is selected from any one or more of a signalling entity (such as a signal peptide), an immune enhancer, a toxin, or a biologically active enzyme, or a sequence coding for any of same.

18. A vector according to any one of the preceding claims wherein the retroviral vector comprises a tumour specific promoter enhancer.

19. A vector according to any one of the preceding claims wherein the vector is a retroviral vector.

20. A method of delivering a nucleotide sequence of interest ("NOI") and/or a product of interest ("POI") encoded by same to a tumour, wherein the NOI and/or POI are delivered to the tumour by use of a vector comprising the NOI and/or expressing the POI; wherein

the NOI and/or the POI is capable of recognising a tumour; wherein the NOI and/or the POI is delivered to the tumour; and wherein the vector is a vector according to any one of the preceding claims.

21. A method according to claim 20 wherein the vector is used to deliver the NOI and/or POI *ex vivo* and/or *in vivo* to the tumour.

22. Use of a vector to deliver a nucleotide sequence of interest ("NOI") and/or a product of interest ("POI") encoded by same to a tumour, wherein the NOI and/or POI are delivered to the tumour by use of the vector which comprises the NOI and/or expresses the POI; wherein the NOI and/or the POI is capable of recognising a tumour when the NOI and/or the POI is delivered to the tumour; and wherein the vector is a vector according to any one of the preceding claims..

23. A use according to claim 22 wherein the vector is used to deliver the NOI and/or POI *ex vivo* and/or *in vivo* to the tumour.

24. A method of treating a subject in need of same, the method comprising delivering a nucleotide sequence of interest ("NOI") and/or a product of interest ("POI") encoded by same to a tumour, wherein the NOI and/or POI are delivered to the tumour by use of a vector comprising the NOI and/or expressing the POI; wherein the NOI and/or the POI is capable of recognising a tumour; wherein the NOI and/or the POI is delivered to the tumour; and wherein the vector is a vector according to any one of the preceding claims.

25. A method according to claim 24 wherein the vector is used to deliver the NOI and/or POI *ex vivo* and/or *in vivo* to the tumour.

26. The use of a genetic vectors to deliver a therapeutic gene encoding a secretable TIP (preferably a TBP) to the interior of a tumour mass.

27. A gene delivery system for targeting one or more genes encoding a TIP (preferably a TBP) to a tumour, comprising a genetic vector encoding a TIP (preferably a TBP) and an *in vivo* gene-delivery system.

28. A method of treating cancer comprising administering at least one TIP (preferably at least one TBP) gene in a gene delivery system according to claim 27 either systemically or directly to the site of a tumour.

29. A gene delivery system for introducing one or more genes encoding a TIP (preferably a TBP) into cells of the haematopoietic (preferably myeloid haematopoietic) cell lineage either *in vivo* or *ex vivo*.

30. A method for treating cancer in a mammal, comprising administering to an individual a gene delivery system according to claim 29 that is capable of expressing a TIP (preferably a TBP) in cells derived from a haematopoietic (preferably myeloid haematopoietic) origin.

31. A genetic vector comprising a therapeutic gene or genes encoding a TIP (preferably a TBP), operably linked to an expression regulatory element selectively functional in a cell type present within a tumour mass.

32. A genetic vector comprising a therapeutic gene or genes is delivered to the interior of the tumour wherein the therapeutic gene encodes a TIP (preferably a TBP), which additionally contains one or more effector domains.

33. A method of treating cancer in a mammal which comprises administering to an individual a combination of a cytokine or a cytokine-encoding gene and one or more TIP (preferably a TBP) genes.

34. The delivery of TIP- (preferably a TBP-) encoding genes to the site of a tumour.

35. A vector comprising (a) a NS coding for a TIP and (b) an NOI which encodes a POI; wherein the TIP is capable of recognising a tumour, such that in use the vector is capable

of delivering the NOI and/or the POI to the tumour; and wherein the TIP and POI are fused to each other.

36. A vector comprising (a) a NS coding for a TIP and (b) an NOI which encodes a POI; wherein the TIP is capable of recognising a tumour, such that in use the vector is capable of delivering the NOI and/or the POI to the tumour; wherein the TIP and POI are fused to each other; and wherein the POI is capable of being secreted.

37. Use of a vector according to any one of the preceding claims as an *in situ* production factory of any one or more of the NS, NOI, POI and TIP.

38. Use of a vector according to any one of the preceding claims when present in a cell to deliver any one or more of the NS, NOI, POI and TIP to a neighbouring cell.

39. A vector substantially as described herein.

ADDITIONAL CLAIMS

40. A process for preparing a TBP comprising expressing a NS encoding a TBP in a vector according to claim 5 or any claim dependent thereon.

41. A TBP wherein the TBP is selected from a group consisting of 5T4ScFv.1, 5T4Sab1, 5T4ScFv-IgG, 5T4ScFv-IgE1, B7-1.5T4.1, B7-1.5T4.2, B7-EGF.

42. A TBP obtained by the process of claim 40 or the TBP of claim 41 for subsequent use in a medical application.

43. A TBP according to claim 42 wherein the medical application is a diagnostic application.

44. A TBP according to claim 42 wherein the medical application is a therapeutic application.

45. Use of a TASCM as defined in claim 7 or claim 8 as a prognostic factor and/or a target for cancer therapy.

46. Use of a TASCM according to claim 45 wherein the TASCM is erb-2.

Figure 1A SEQ ID No 1.

```
1    GAGGTCCAGC TTCAGCAGTC TGGACCTGAC CTGGTGAAGC CTGGGGCTTC
      E  V  Q   L  Q  Q  S   G  P  D   L  V  K   P  G  A  S

51   AGTGAAGATA TCCTGCAAGG CTTCTGGTTA CTCATTCACT GGCTACTACA
      V  K  I   S  C  K   A  S  G  Y   S  F  T   G  Y  Y

101  TGCACTGGGT GAAGCAGAGC CATGGAAAGA GCCTTGAGTG GATTGGACGT
     M  H  W  V   K  Q  S   H  G  K   S  L  E  W   I  G  R

151  ATTAATCCTA ACAATGGTGT TACTCTCTAC AACCAGAAAT TCAAGGACAA
      I  N  P   N  N  G  V   T  L  Y   N  Q  K   F  K  D  K

201  GGCCATATTA ACTGTAGACA AGTCATCCAC CACAGCCTAC ATGGAGCTCC
      A  I  L   T  V  D   K  S  S  T   T  A  Y   M  E  L

251  GCAGCCTGAC ATCTGAGGAC TCTGCGGTCT ATTACTGTGC AAGATCTACT
     R  S  L  T   S  E  D   S  A  V   Y  Y  C  A   R  S  T

301  ATGATTACGA ACTATGTTAT GGACTACTGG GGTCAAGTAA CCTCAGTCAC
      M  I  T   N  Y  V  M   D  Y  W   G  Q  V   T  S  V  T

351  CGTCTCCTCA GGTGGTGGTG GGAGCGGTGG TGGCGGCACT GGCGGCGGCG
      V  S  S   G  G  G   G  S  G  G   G  G  T   G  G  G

401  GATCTAGTAT TGTGATGACC CAGACTCCCA CATTCCTGCT TGTTTCAGCA
     G  S  S  I   V  M  T   Q  T  P   T  F  L  L   V  S  A

451  GGAGACAGGG TTACCATAAC CTGCAAGGCC AGTCAGAGTG TGAGTAATGA
      G  D  R   V  T  I  T   C  K  A   S  Q  S   V  S  N  D

501  TGTAGDTTGG TACCAACAGA AGCCAGGGCA GTCTCCTACA CTGCTCATAT
      V  A  W   Y  Q  Q   K  P  G  Q   S  P  T   L  L  I

551  CCTATACATC CAGTCGCTAC GCTGGAGTCC CTGATCGCTT CATTGGCAGT
     S  Y  T  S   S  R  Y   A  G  V   P  D  R  F  I  G  S

601  GGATATGGGA CGGATTTCAC TTTCACCATC AGCACTTTGC AGGCTGAAGA
      G  Y  G   T  D  F  T   F  T  I   S  T  L   Q  A  E  D

651  CCTGGCAGTT TATTTCTGTC AGCAAGATTA TAATTCTCCT CCGACGTTCG
      L  A  V   Y  F  C   Q  Q  D  Y   N  S  P   P  T  F

701  GTGGAGGCAC CAAGCTGGAA ATCAAACGG
     G  G  G  T   K  L  E   I  K  R
```

Figure 1B SEQ ID No 2.

```
1    AAGCTTCCAC CATGGGATGG AGCTGTATCA TCCTCTTCTT GGTAGCAACA
       A  S  T   M  G  W   S  C  I   I  L  F  L   V  A  T

51   GCTACAGGTG TCCACTCCGA GGTCCAGCTT CAGCAGTCTG GACCTGACCT
      A  T  G   V  H  S  E   V  Q  L   Q  Q  S   G  P  D  L

101  GGTGAAGCCT GGGGCTTCAG TGAAGATATC CTGCAAGGCT TCTGGTTACT
       V  K  P   G  A  S   V  K  I  S   C  K  A   S  G  Y

151  CATTCACTGG CTACTACATG CACTGGGTGA AGCAGAGCCA TGGAAAGAGC
      S  F  T  G   Y  Y  M   H  W  V   K  Q  S  H   G  K  S

201  CTTGAGTGGA TTGGACGTAT TAATCCTAAC AATGGTGTTA CTCTCTACAA
       L  E  W   I  G  R  I   N  P  N   N  G  V   T  L  Y  N

251  CCAGAAATTC AAGGACAAGG CCATATTAAC TGTAGACAAG TCATCCACCA
       Q  K  F   K  D  K   A  I  L  T   V  D  K   S  S  T

301  CAGCCTACAT GGAGCTCCGC AGCCTGACAT CTGAGGACTC TGCGGTCTAT
      T  A  Y  M   E  L  R   S  L  T   S  E  D  S   A  V  Y

351  TACTGTGCAA GATCTACTAT GATTACGAAC TATGTTATGG ACTACTGGGG
       Y  C  A   R  S  T  M   I  T  N   Y  V  M   D  Y  W  G

401  TCAAGTAACC TCAGTCACCG TCTCCTCAGG TGGTGGTGGG AGCGGTGGTG
       Q  V  T   S  V  T   V  S  S  G   G  G  G   S  G  G

451  GCGGCACTGG CGGCGGCGGA TCTAGTATTG TGATGACCCA GACTCCCACA
       G  G  T   G  G  G   S  S  I   V  M  T  Q   T  P  T

501  TTCCTGCTTG TTTCAGCAGG AGACAGGGTT ACCATAACCT GCAAGGCCAG
       F  L  L   V  S  A  G   D  R  V   T  I  T  C   K  A  S

551  TCAGAGTGTG AGTAATGATG TAGCTTGGTA CCAACAGAAG CCAGGGCAGT
       Q  S  V   S  N  D   V  A  W  Y   Q  Q  K   P  G  Q

601  CTCCTACACT GCTCATATCC TATACATCCA GTCGCTACGC TGGAGTCCCT
      S  P  T  L   L  I  S   Y  T  S   S  R  Y  A   G  V  P

651 GATCGCTTCA TTGGCAGTGG ATATGGGACG GATTTCACTT TCACCATCAG
      D  R  F   I  G  S  G   Y  G  T   D  F  T   F  T  I  S

701  CACTTTGCAG GCTGAAGACC TGGCAGTTTA TTTCTGTCAG CAAGATTATA
       T  L  Q   A  E  D   L  A  V  Y   F  C  Q   Q  D  Y
```

```
751  ATTCTCCTCC GACGTTCGGT GGAGGCACCA AGCTGGAAAT CAAACGGGCC
       N  S  P  P  T  F  G  G  G  T  K  L  E  I  K  R  A

801  TCCACCAAGG GCCCATCGGT CTTCCCCCTG GCACCCTCCT CCAAGAGCAC
       S  T  K  G  P  S  V  F  P  L  A  P  S  S  K  S  T

851  CTCTGGGGGC ACAGCGGCCC TGGGCTGCCT GGTCAAGGAC TACTTCCCCG
       S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P

901  AACCGGTGAC GGTGTCGTGG AACTCAGGCG CCCTGACCAG CGGCGTGCAC
       E  P  V  T  V  S  W  N  S  G  A  L  T  S  G  V  H

951  ACCTTCCCGG CTGTCCTACA GTCCTCAGGA CTCTACTCCC TCAGCAGCGT
       T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V

1001 GGTGACCGTG CCCTCCAGCA GCTTGGGCAC CCAGACCTAC ATCTGCAACG
       V  T  V  P  S  S  S  L  G  T  Q  T  Y  I  C  N

1051 TGAATCACAA GCCCAGCAAC ACCAAGGTGG ACAAGAAAGT TGAGCCCAAA
       V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K

1101 TCTTGTGACA AAACTCACAC ATGCCCACCG TGCCCAGCAC CTGAACTCCT
       S  C  D  K  T  H  T  C  P  P  C  P  A  P  E  L  L

1151 GGGGGGACCG TCAGTCTTCC TCTTCCCCCC AAAACCCAAG GACACCCTCA
       G  G  P  S  V  F  L  F  P  P  K  P  K  D  T  L

1201 TGATCTCCCG GACCCCTGAG GTCACATGCG TGGTGGTGGA CGTGAGCCAC
       M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S  H

1251 GAAGACCCTG AGGTCAAGTT CAACTGGTAC GTGGACGGCG TGGAGGTGCA
       E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H

1301 TAATGCCAAG ACAAAGCCGC GGGAGGAGCA GTACAACAGC ACGTACCGTG
       N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R

1351 TGGTCAGCGT CCTCACCGTC CTGCACCAGG ACTGGCTGAA TGGCAAGGAG
       V  V  S  V  L  T  V  L  H  Q  D  W  L  N  G  K  E

1401 TACAAGTGCA AGGTCTCCAA CAAAGCCCTC CCAGCCCCCA TCGAGAAAAC
       Y  K  C  K  V  S  N  K  A  L  P  A  P  I  E  K  T

1451 CATCTCCAAA GCCAAAGGGC AGCCCCGAGA ACCACAGGTG TACACCCTGC
       I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L

1501 CCCCATCCCG GGATGAGCTG ACCAAGAACC AGGTCAGCCT GACCTGCCTG
       P  P  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L
```

```
1551 GTCAAAGGCT TCTATCCCAG CGACATCGCC GTGGAGTGGG AGAGCAATGG
      V  K  G   F  Y  P  S   D  I  A   V  E  W   E  S  N  G

1601 GCAGCCGGAG AACAACTACA AGACCACGCC TCCCGTGCTG GACTCCGACG
       Q  P  E   N  N  Y   K  T  T  P   P  V  L   D  S  D

1651 GCTCCTTCTT CCTCTACAGC AAGCTCACCG TGGACAAGAG CAGGTGGCAG
     G  S  F  F   L  Y  S  K  L  T  V   D  K  S   R  W  Q

1701 CAGGGGAACG TCTTCTCATG CTCCGTGATG CATGAGGCTC TGCACAACCA
      Q  G  N   V  F  S  C   S  V  M   H  E  A   L  H  N  H

1751 CTACACGCAG AAGAGCCTCT CCCTGTCTCC GGGTAAATGA GTGCGACGGC
       Y  T  Q   K  S  L   S  L  S  P   G  K  -   V  R  R

1801 CAAGCTT
     P  S
```

Figure 2. SEQ ID No3.

```
ATGGGCCACA CACGGAGGCA GGGAACATCA CCATCCAAGT GTCCATACCT      50
 M  G  H   T  R  R  Q   G  T  S   P  S  K   C  P  Y  L

CAATTTCTTT CAGCTCTTGG TGCTGGCTGG TCTTTCTCAC TTCTGTTCAG     100
  N  F  F   Q  L  L   V  L  A  G   L  S  H   F  C  S

GTGTTATCCA CGTGACCAAG GAAGTGAAAG AAGTGGCAAC GCTGTCCTGT     150
G  V  I  H   V  T  K   E  V  K   E  V  A  T   L  S  C

GGTCACAATG TTTCTGTTGA AGAGCTGGCA CAAACTCGCA TCTACTGGCA     200
 G  H  N   V  S  V  E   E  L  A   Q  T  R   I  Y  W  Q

AAAGGAGAAG AAAATGGTGC TGACTATGAT GTCTGGGGAC ATGAATATAT     250
  K  E  K   K  M  V   L  T  M  M   S  G  D   M  N  I

GGCCCGAGTA CAAGAACCGG ACCATCTTTG ATATCACTAA TAACCTCTCC     300
W  P  E  Y   K  N  R   T  I  F   D  I  T  N   N  L  S

ATTGTGATCC TGGCTCTGCG CCCATCTGAC GAGGGCACAT ACGAGTGTGT     350
 I  V  I   L  A  L  R   P  S  D   E  G  T   Y  E  C  V

TGTTCTGAAG TATGAAAAAG ACGCTTTCAA GCGGGAACAC CTGGCTGAAG     400
  V  L  K   Y  E  K   D  A  F  K   R  E  H   L  A  E

TGACGTTATC AGTCAAAGCT GACTTCCCTA CACCTAGTAT ATCTGACTTT     450
V  T  L  S   V  K  A   D  F  P   T  P  S  I   S  D  F

GAAATTCCAA CTTCTAATAT TAGAAGGATA ATTTGCTCAA CCTCTGGAGG     500
 E  I  P   T  S  N  I   R  R  I   I  C  S   T  S  G  G

TTTTCCAGAG CCTCACCTCT CCTGGTTGGA AAATGGAGAA GAATTAAATG     550
  F  P  E   P  H  L   S  W  L  E   N  G  E   E  L  N

CCATCAACAC AACAGTTTCC CAAGATCCTG AAACTGAGCT CTATGCTGTT     600
A  I  N  T   T  V  S   Q  D  P   E  T  E  L   Y  A  V

AGCAGCAAAC TGGATTTCAA TATGACAACC AACCACAGCT TCATGTGTCT     650
 S  S  K   L  D  F  N   M  T  T   N  H  S   F  M  C  L

CATCAAGTAT GGACATTTAA GAGTGAATCA GACCTTCAAC TGGAATACAA     700
  I  K  Y   G  H  L   R  V  N  Q   T  F  N   W  N  T
```

```
CCAAGCAAGA GCATTTTCCT GATGGAGGCG GGGGATCCGA GGTCCAGCTT      750
T  K  Q  E   H  F  P   D  G  G   G  G  S  E   V  Q  L

CAGCAGTCTG GACCTGACCT GGTGAAGCCT GGGGCTTCAG TGAAGATATC      800
 Q  Q  S   G  P  D  L   V  K  P   G  A  S   V  K  I  S

CTGCAAGGCT TCTGGTTACT CATTCACTGG CTACTACATG CACTGGGTGA      850
  C  K  A   S  G  Y   S  F  T  G   Y  Y  M   H  W  V

AGCAGAGCCA TGGAAAGAGC CTTGAGTGGA TTGGACGTAT TAATCCTAAC      900
K  Q  S  H   G  K  S   L  E  W   I  G  R  I   N  P  N

AATGGTGTTA CTCTCTACAA CCAGAAATTC AAGGACAAGG CCATATTAAC      950
 N  G  V   T  L  Y  N   Q  K  F   K  D  K   A  I  L  T

TGTAGACAAG TCATCCACCA CAGCCTACAT GGAGCTCCGC AGCCTGACAT     1000
  V  D  K   S  S  T   T  A  Y  M   E  L  R   S  L  T

CTGAGGACTC TGCGGTCTAT TACTGTGCAA GATCTACTAT GATTACGAAC     1050
S  E  D  S   A  V  Y   Y  C  A   R  S  T  M   I  T  N

TATGTTATGG ACTACTGGGG TCAAGTAACC TCAGTCACCG TCTCCTCAGG     1100
 Y  V  M   D  Y  W  G   Q  V  T   S  V  T   V  S  S  G

TGGTGGTGGG AGCGGTGGTG GCGGCACTGG CGGCGGCGGA TCTAGTATTG     1150
  G  G  G   S  G  G   G  G  T  G   G  G  G   S  S  I

TGATGACCCA GACTCCCACA TTCCTGCTTG TTTCAGCAGG AGACAGGGTT     1200
V  M  T  Q   T  P  T   F  L  L   V  S  A  G   D  R  V

ACCATAACCT GCAAGGCCAG TCAGAGTGTG AGTAATGATG TAGCTTGGTA     1250
 T  I  T   C  K  A  S   Q  S  V   S  N  D   V  A  W  Y

CCAACAGAAG CCAGGGCAGT CTCCTACACT GCTCATATCC TATACATCCA     1300
  Q  Q  K   P  G  Q   S  P  T  L   L  I  S   Y  T  S

GTCGCTACGC TGGAGTCCCT GATCGCTTCA TTGGCAGTGG ATATGGGACG     1350
S  R  Y  A   G  V  P   D  R  F   I  G  S  G   Y  G  T

GATTTCACTT TCACCATCAG CACTTTGCAG GCTGAAGACC TGGCAGTTTA     1400
 D  F  T   F  T  I  S   T  L  Q   A  E  D   L  A  V  Y

TTTCTGTCAG CAAGATTATA ATTCTCCTCC GACGTTCGGT GGAGGCACCA     1450
  F  C  Q   Q  D  Y   N  S  P  P   T  F  G   G  G  T
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K L E I K .

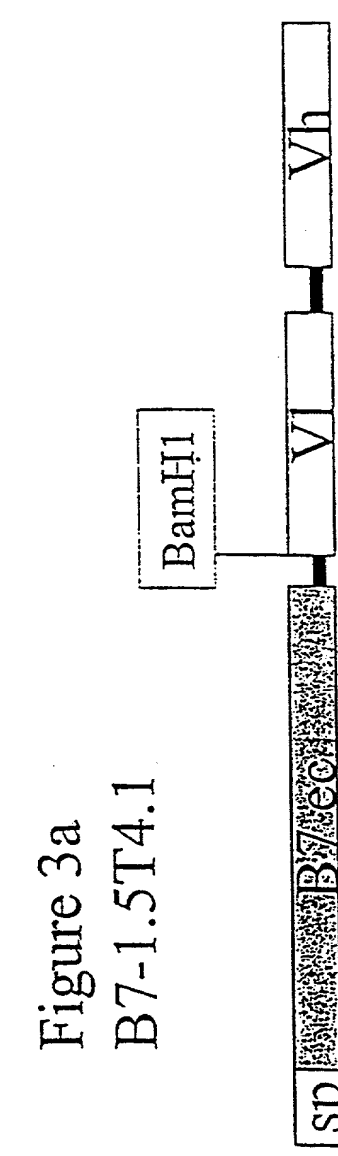

Figure 3a
B7-1.5T4.1

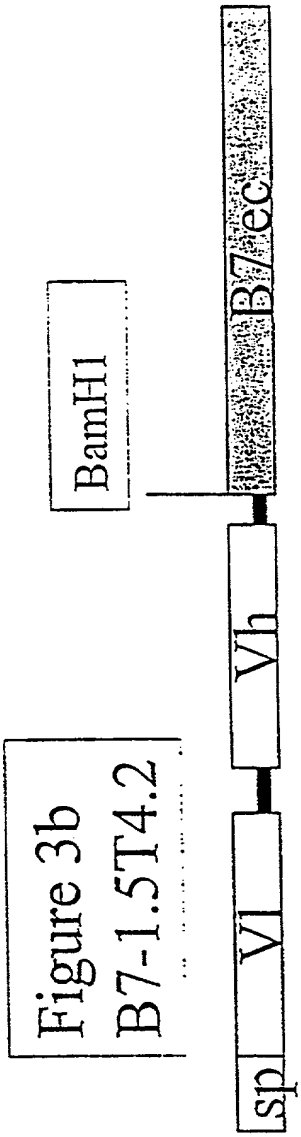

Figure 3b
B7-1.5T4.2

Figure 4 seq id No. 4.

Molecule Name-: B7-2(1-241) 738 bps DNA Linear
Sequence Printed: 1-738 (Full) Date Printed 02 Jun 1997
Description:

```
1    ATGGGACTGA GTAACATTCT CTTTGTGATG GCCTTCCTGC TCTCTGGTGC
       M  G  L  S  N  I  L  F  V  M  A  F  L  L  S  G  A

51   TGCTCCTCTG AAGATTCAAG CTTATTTCAA TGAGACTGCA GACCTGCCAT
       A  P  L  K  I  Q  A  Y  F  N  E  T  A  D  L  P

101  GCCAATTTGC AAACTCTCAA AACCAAAGCC TGAGTGAGCT AGTAGTATTT
       C  Q  F  A  N  S  Q  N  Q  S  L  S  E  L  V  V  F

151  TGGCAGGACC AGGAAAACTT GGTTCTGAAT GAGGTATACT TAGGCAAAGA
       W  Q  D  Q  E  N  L  V  L  N  E  V  Y  L  G  K  E

201  GAAATTTGAC AGTGTTCATT CCAAGTATAT GGGCCGCACA AGTTTTGATT
       K  F  D  S  V  H  S  K  Y  M  G  R  T  S  F  D

251  CGGACAGTTG GACCCTGAGA CTTCACAATC TTCAGATCAA GGACAAGGGC
       S  D  S  W  T  L  R  L  H  N  L  Q  I  K  D  K  G

301  TTGTATCAAT GTATCATCCA TCACAAAAAG CCCACAGGAA TGATTCGCAT
       L  Y  Q  C  I  I  H  H  K  K  P  T  G  M  I  R  I

351  CCACCAGATG AATTCTGAAC TGTCAGTGCT TGCTAACTTC AGTCAACCTG
       H  Q  M  N  S  E  L  S  V  L  A  N  F  S  Q  P

401  AAATAGTACC AATTTCTAAT ATAACAGAAA ATGTGTACAT AAATTTGACC
       E  I  V  P  I  S  N  I  T  E  N  V  Y  I  N  L  T

451  TGCTCATCTA TACACGGTTA CCCAGAACCT AAGAAGATGA GTGTTTTGCT
       C  S  S  I  H  G  Y  P  E  P  K  K  M  S  V  L  L

501  AAGAACCAAG AATTCAACTA TCGAGTATGA TGGTATTATG CAGAAATCTC
       R  T  K  N  S  T  I  E  Y  D  G  I  M  Q  K  S

551  AAGATAATGT CACAGAACTG TACGACGTTT CCATCAGCTT GTCTGTTTCA
       Q  D  N  V  T  E  L  Y  D  V  S  I  S  L  S  V  S

601  TTCCCTGATG TTACGAGCAA TATGACCATC TTCTGTATTC TGGAAACTGA
       F  P  D  V  T  S  N  M  T  I  F  C  I  L  E  T  D

651  CAAGACGCGG CTTTTATCTT CACCTTTCTC TATAGAGCTT GAGGACCCTC
       K  T  R  L  L  S  S  P  F  S  I  E  L  E  D  P
```

```
701  AGCCTCCCCC AGACCACATT CCTGGAGGCG GGGGATCC
       Q  P  P  P   D  H  I   P  G  G   G  G  S
```

#4

DECLARATION AND POWER OF ATTORNEY- USA PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled VECTOR; the specification of which was Internationally filed on **June 4, 1998** as International Application No. **PCT/GB98/01627**, the U.S. National Phase of which has been assigned **SERIAL NO. 09/445,375.**

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above;

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56;

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)			Priority Claimed
No.: **9711579.4**	Country: **United Kingdom**	Date Filed: **June 4, 1997**	**Yes**
No.: **9713150.2**	Country: **United Kingdom**	Date Filed: **June 20, 1997**	**Yes**
No.: **9714230.1**	Country: **United Kingdom**	Date Filed: **July 4, 1997**	**Yes**

POWER OF ATTORNEY: I hereby appoint the registrants of Knobbe, Martens, Olson & Bear, LLP, 620 Newport Center Drive, Sixteenth Floor, Newport Beach, California 92660, Telephone (949) 760-0404, **Customer No. 20,995**.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

---

1 - ∞ Full name of first inventor: **Susan Mary Kingsman**

Inventor's signature _____

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	KINGSMAN et al.
Int'l. App. No.	:	PCT/GB98/01627
Int'l. Filed	:	June 4, 1998
For	:	VECTOR
Examiner	:	Unknown

ESTABLISHMENT OF RIGHT OF ASSIGNEE TO TAKE ACTION
AND
REVOCATION AND POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

The undersigned is empowered to act on behalf of the assignee below (the "Assignee"). A true copy of the original Assignment of the above-captioned application from the inventors to the Assignee is attached hereto. This Assignment represents the entire chain of title of this invention from the Inventors to the Assignee.

I declare that all statements made herein are true, and that all statements made upon information and belief are believed to be true, and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that willful, false statements may jeopardize the validity of the application, or any patent issuing thereon.

The undersigned hereby revokes any previous powers of attorney in the subject application, and hereby appoints the registrants of Knobbe, Martens, Olson & Bear, LLP, 620 Newport Center Drive, Sixteenth Floor, Newport Beach, California 92660, Telephone (949) 760-0404, **Customer No. 20,995**, as its attorneys with full power of substitution and revocation to prosecute this application and to transact all business in the U.S. Patent and

Trademark Office connected herewith. This appointment is to be to the exclusion of the inventors and his attorneys in accordance with the provisions of 37 C.F.R. § 3.71.

Please use **Customer No. 20,995** for all communications.

OXFORD BIOMEDICA (UK) LIMITED

Dated: 20 February 2000

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H:\DOCS\JAH\JAH-2040.DOC
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SEQUENCE LISTING

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Ellard, F.
Carroll, Miles

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<223> Description of Artificial Sequence: DNA

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gaggtccagc ttcagcagtc tggacctgac ctggtgaagc ctggggcttc vsgdvkgasa 60
gtgaagatat cctgcaaggc ttctggttac tcattcactg gctactacav ksckasgyst 120
gyytgcactg ggtgaagcag agccatggaa agagccttga gtggattgga cgtmhwvksh 180
gkswgratta atcctaacaa tggtgttact ctctacaacc agaaattcaa ggacaannng 240
vtynkkdkgg ccatattaac tgtagacaag tcatccacca cagcctacat ggagctccat 300
vdkssttaym gcagcctgac atctgaggac tctgcggtct attactgtgc aagatctact 360
rstsdsavyy carstatgat tacgaactat gttatggact actggggtca agtaacctca 420
gtcacmtnyv mdywgvtsvt cgtctcctca ggtggtggtg ggagcggtgg tggcggcact 480
ggcggcggcg vssgggggsgg ggtggggatc tagtattgtg atgacccaga ctcccacatt 540
cctgcttgtt tcagcagssv mtttvsagga gacagggtta ccataacctg caaggccagt 600
cagagtgtga gtaatgagdr vttckassvs ndtgtagdtt ggtaccaaca gaagccaggg 660
cagtctccta cactgctcat atvawykgst cctatacatc cagtcgctac gctggagtcc 720
ctgatcgctt cattggcagt sytssryagv drgsggatat gggacggatt tcactttcac 780
catcagcact ttgcaggctg aagagygtdt tstadcctgg cagtttattt ctgtcagcaa 840
gattataatt ctcctccgac gttcgavycd ynstgtggag gcaccaagct ggaaatcaaa 900
cgggggtkkr 910

<210> 2
<211> 2239
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: cDNA

<400> 2
aagcttccac catgggatgg agctgtatca tcctcttctt ggtagcaaca astmgwscva 60
tgctacaggt gtccactccg aggtccagct tcagcagtct ggacctgacc tatgvhsvsg 120
dggtgaagcc tggggcttca gtgaagatat cctgcaaggc ttctggttac tvkgasvksc 180
kasgycattc actggctact acatgcactg ggtgaagcag agccatggaa agagcstgyy 240

mhwvkshgks cttgagtgga ttggacgtat taatcctaac aatggtgtta ctctctacaa 300
wgrnnngvty nccagaaatt caaggacaag gccatattaa ctgtagacaa gtcatccacc 360
akkdkatvdk sstcagccta catggagctc cgcagcctga catctgagga ctctgcggtc 420
tattaymrst sdsavytact gtgcaagatc tactatgatt acgaactatg ttatggacta 480
ctggggycar stmtnyvmdy wgtcaagtaa cctcagtcac cgtctcctca ggtggtggtg 540
ggagcggtgg tgvtsvtvss ggggsgggcg gcactggcgg cggcggatct agtattgtga 600
tgacccagac tcccacaggt ggggssvmtt tttcctgctt gtttcagcag gagacagggt 660
taccataacc tgcaaggcca gvsagdrvtt ckastcagag tgtgagtaat gatgtagctt 720
ggtaccaaca gaagccaggg cagtsvsndv awykgctcct acactgctca tatcctatac 780
atccagtcgc tacgctggag tccctstsyt ssryagvgat cgcttcattg gcagtggata 840
tgggacggat ttcactttca ccatcagdrg sgygtdttsc actttgcagg ctgaagacct 900
ggcagtttat ttctgtcagc aagattatat adavycdyat tctcctccga cgttcggtgg 960
aggcaccaag ctggaaatca aacgggccns tgggtkkrat ccaccaaggg cccatcggtc 1020
ttccccctgg caccctcctc caagagcacs tkgsvassks tctctggggg cacagcggcc 1080
ctgggctgcc tggtcaagga ctacttcccc gsggtaagcv kdyaaccggt gacggtgtcg 1140
tggaactcag gcgccctgac cagcggcgtg cacvtvswns gatsgvhacc ttcccggctg 1200
tcctacagtc ctcaggactc tactccctca gcagcgttav ssgysssvgg tgaccgtgcc 1260
ctccagcagc ttgggcaccc agacctacat ctgcaacgvt vsssgttycn tgaatcacaa 1320
gcccagcaac accaaggtgg acaagaaagt tgagcccaaa vnhksntkvd kkvktcttgt 1380
gacaaaactc acacatgccc accgtgccca gcacctgaac tcctscdkth tccaggggg 1440
accgtcagtc ttcctcttcc ccccaaaacc caaggacacc ctcaggsvkk dttgatctcc 1500
cggacccctg aggtcacatg cgtggtggtg gacgtgagcc acmsrtvtcv vvdvshgaag 1560
accctgaggt caagttcaac tggtacgtgg acggcgtgga ggtgcadvkn wyvdgvvhta 1620
atgccaagac aaagccgcgg gaggagcagt acaacagcac gtaccgtgna ktkrynstyr 1680
tggtcagcgt cctcaccgtc ctgcaccagg actggctgaa tggcaaggag vvsvtvhdwn 1740
gktacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa acykckvsnk 1800
aaktcatctc caaagccaaa gggcagcccc gagaaccaca ggtgtacacc ctgcskakgr 1860
vytccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc ctgsrdtknv 1920
stcgtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tggvkgysda 1980
vwsnggcagc cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacgnnykt 2040
tvdsdgctcc ttcttcctct acagcaagct caccgtggac aagagcaggt ggcaggsysk 2100
tvdksrwcag gggaacgtct tctcatgctc cgtgatgcat gaggctctgc acaaccagnv 2160
scsvmhahnh ctacacgcag aagagcctct ccctgtctcc gggtaaatga gtgcgacggc 2220
ytksssgkvr rcaagctts 2239

<210> 3
<211> 1809
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA

<400> 3
atgggccaca cacggaggca gggaacatca ccatccaagt gtccatacct mghtrrgtss 60
kcycaatttc tttcagctct tggtgctggc tggtctttct cacttctgtt cagnvagshc 120
sgtgttatcc acgtgaccaa ggaagtgaaa gaagtggcaa cgctgtcctg tgvhvtkvkv 180
atscggtcac aatgtttctg ttgaagagct ggcacaaact cgcatctact ggcaghnvsv 240
atrywaaagg agaagaaaat ggtgctgact atgatgtctg gggacatgaa tatatkkkmv 300
tmmsgdmngg cccgagtaca agaaccggac catctttgat atcactaata acctctccwy 360
knrtdtnnsa ttgtgatcct ggctctgcgc ccatctgacg agggcacata cgagtgtgtv 420
arsdgtycvt gttctgaagt atgaaaaaga cgctttcaag cgggaacacc tggctgaagv 480
kykdakrhat gacgttatca gtcaaagctg acttccctac acctagtata tctgactttv 540
tsvkadtssd gaaattccaa cttctaatat tagaaggata atttgctcaa cctctggagg 600
tsnrrcstsg gttttccaga gcctcacctc tcctggttgg aaaatggaga agaattaaat 660
ghswngncca tcaacacaac agtttcccaa gatcctgaaa ctgagctcta tgctgttant 720
tvsdtyavag cagcaaactg gatttcaata tgacaaccaa ccacagcttc atgtgtctss 780

kdnmttnhsm ccatcaagta tggacattta agagtgaatc agaccttcaa ctggaataca 840
akyghrvntn wntccaagca agagcatttt cctgatggag gcgggggatc cgaggtccag 900
ctttkhdggg gsvcagcagt ctggacctga cctggtgaag cctggggctt cagtgaagat 960
atcsgdvkga svksctgcaa ggcttctggt tactcattca ctggctacta catgcactgg 1020
gtgackasgy stgyymhwva gcagagccat ggaaagagcc ttgagtggat tggacgtatt 1080
aatcctaack shgkswgrnn aatggtgtta ctctctacaa ccagaaattc aaggacaagg 1140
ccatattaac ngvtynkkdk attgtagaca agtcatccac cacagcctac atggagctcc 1200
gcagcctgac atvdkssttа ymrstctgag gactctgcgg tctattactg tgcaagatct 1260
actatgatta cgaacsdsav yycarstmtn tatgttatgg actactgggg tcaagtaacc 1320
tcagtcaccg tctcctcagg yvmdywgvts vtvssgtggt ggtgggagcg gtggtggcgg 1380
cactggcggc ggcggatcta gtattggggs ggggtggggs stgatgaccc agactcccac 1440
attcctgctt gtttcagcag gagacagggt tvmtttvsag drvaccataa cctgcaaggc 1500
cagtcagagt gtgagtaatg atgtagcttg gtattckass vsndvawycc aacagaagcc 1560
agggcagtct cctacactgc tcatatccta tacatccakg stsytsgtcg ctacgctgga 1620
gtccctgatc gcttcattgg cagtggatat gggacgsrya gvdrgsgygt gatttcactt 1680
tcaccatcag cactttgcag gctgaagacc tggcagttta dttstadavy tttctgtcag 1740
caagattata attctcctcc gacgttcggt ggaggcacca cdynstgggt agctggaaat 1800
caaataakk 1809

<210> 4
<211> 887
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA

<400> 4
atgggactga gtaacattct ctttgtgatg gccttcctgc tctctggtgc mgsnvmasga 60
tgctcctctg aagattcaag cttatttcaa tgagactgca gacctgccat akayntadgc 120
caatttgcaa actctcaaaa ccaaagcctg agtgagctag tagtatttca nsnssvvtgg 180
caggaccagg aaaacttggt tctgaatgag gtatacttag gcaaagawdn vnvygkgaaa 240
tttgacagtg ttcattccaa gtatatgggc cgcacaagtt ttgattkdsv hskymgrtsd 300
cggacagttg gaccctgaga cttcacaatc ttcagatcaa ggacaagggc sdswtrhnkd 360
kgttgtatca atgtatcatc catcacaaaa agcccacagg aatgattcgc atychhkktg 420
mrccaccaga tgaattctga actgtcagtg cttgctaact tcagtcaacc tghmnssvan 480
saaatagtac caatttctaa tataacagaa aatgtgtaca taaatttgac cvsntnvynt 540
tgctcatcta tacacggtta cccagaacct aagaagatga gtgttttgct csshgykkms 600
vaagaaccaa gaattcaact atcgagtatg atggtattat gcagaaatct crtknstydg 660
mksaagataa tgtcacagaa ctgtacgacg tttccatcag cttgtctgtt tcadnvtydv 720
sssvsttccc tgatgttacg agcaatatga ccatcttctg tattctggaa actgadvtsn 780
mtctdcaaga cgcggctttt atcttcacct ttctctatag agcttgagga ccctcktrss 840
sdagcctccc ccagaccaca ttcctggagg cgggggatcc dhggggs 887

<210> 5
<211> 1518
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA

<400> 5
atggcttgca attgtcagtt gatgcaggat acaccactcc tcaagtttcc atgtccaagg 60
ctcattcttc tctttgtgct gctgattcgt ctttcacaag tgtcttcaga tgttgatgaa 120
caactgtcca agtcagtgaa agataaggta ttgctgcctt gccgttacaa ctctccgcat 180
gaagatgagt ctgaagaccg aatctactgg caaaaacatg acaaagtggt gctgtctgtc 240

attgctggga aactaaaagt gtggcccgag tataagaacc ggactttata tgacaacact 300
acctactctc ttatcatcct gggcctggtc ctttcagacc ggggcacata cagctgtgtc 360
gttcaaaaga aggaaagagg aacgtatgaa gttaaacact tggctttagt aaagttgtcc 420
atcaaagctg acttctctac ccccaacata actgagtctg gaaacccatc tgcagacact 480
aaaaggatta cctgctttgc ttccgggggt ttcccaaagc ctcgcttctc ttggttggaa 540
aatggaagag aattacctgg catcaatacg acaatttccc aggatcctga atctgaattg 600
tacaccatta gtagccaact agatttcaat acgactcgca accacaccat taagtgtctc 660
attaaatatg gagatgctca cgtgtcagag gacttcacct gggaaaaacc cccagaagag 720
cctcctgata gcaagcccgg gggtggtggg agcggtggtg gcggcagtgg cggcggcgga 780
actagtgagg tccagcttca gcagtctgga cctgacctgg tgaagcctgg ggcttcagtg 840
aagatatcct gcaaggcttc tggttactca ttcactggct actacatgca ctgggtgaag 900
cagagccatg gaaagagcct tgagtggatt ggacgtatta atcctaacaa tggtgttact 960
ctctacaacc agaaattcaa ggacaaggcc atattaactg tagacaagtc atccaccaca 1020
gcctacatgg agctccgcag cctgacatct gaggactctg cggtctatta ctgtgcaaga 1080
tctactatga ttacgaacta tgttatggac tactggggtc aagtaacttc agtcaccgtc 1140
tcttcaggtg gtggtgggag cggtggtggc ggcactggcg gcggcggatc tagtattgtg 1200
atgacccaga ctcccacatt cctgcttgtt tcagcaggag acagggttac ataacctgc 1260
aaggccagtc agagtgtgag taatgatgta gcttggtacc aacagaagcc agggcagtct 1320
cctacactgc tcatatccta tacatccagt cgctacgctg gagtccctga tcgcttcatt 1380
ggcagtggat atgggacgga tttcactttc accatcagca ctttgcaggc tgaagacctg 1440
gcagtttatt tctgtcagca agattataat tctcctccga cgttcggtgg aggcaccaag 1500
ctggaaatca aacggtaa 1518

<210> 6
<211> 2090
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA

<400> 6
ctcgagccac catgggatgg agctgtatca tcctcttctt ggtagcaaca gctacaggtg 60
tccactccga ggtccagctg cagcagtctg gacctgacct ggtgaagcct ggggcttcag 120
tgaagatatc ctgcaaggct tctggttact cattcactgg ctactacatg cactgggtga 180
agcagagcca tggaaagagc cttgagtgga ttggacgtat taatcctaac aatggtgtta 240
ctctctacaa ccagaaattc aaggacaagg ccatattaac tgtagacaag tcatccacca 300
cagcctacat ggagctccgc agcctgacat ctgaggactc tgcggtctat tactgtgcaa 360
gatctactat gattacgaac tatgttatgg actactgggg tcaagtaact tcagtcaccg 420
tctcttcagg tggtggtggg agcggtggtg gcggcactgg cggcggcgga tctagtattg 480
tgatgaccca gactcccaca ttcctgcttg tttcagcagg agacagggtt accataacct 540
gcaaggccag tcagagtgtg agtaatgatg tagcttggta ccaacagaag ccagggcagt 600
ctcctacact gctcatatcc tatacatcca gtcgctacgc tggagtccct gatcgcttca 660
ttggcagtgg atatgggacg gatttcactt tcaccatcag cactttgcag gctgaagacc 720
tggcagttta tttctgtcag caagattata attctcctcc gacgttcggt ggaggcacca 780
agcttgaaat caaacgggcc tccacacaga gcccatccgt cttccccttg acccgctgct 840
gcaaaaacat tccctccaat gccacctccg tgactctggg ctgcctggcc acgggctact 900
tcccggagcc ggtgatggtg acctgggaca caggctccct caacgggaca actatgacct 960
taccagccac caccctcacg ctctctggtc actatgccac catcagcttg ctgaccgtct 1020
cgggtgcgtg ggccaagcag atgttcacct gccgtgtggc acacactcca tcgtccacag 1080
actgggtcga caacaaaacc ttcagcgtct gctccaggga cttcaccccg cccaccgtga 1140
agatcttaca gtcgtcctgc gacggcggcg ggcacttccc cccgaccatc cagctcctgt 1200
gcctcgtctc tgggtacacc ccagggacta tcaacatcac ctggctggag gacgggcagg 1260
tcatggacgt ggacttgtcc accgcctcta ccacgcagga gggtgagctg gcctccacac 1320
aaagcgagct caccctcagc cagaagcact ggctgtcaga ccgcacctac acctgccagg 1380
tcacctatca aggtcacacc tttgaggaca gcaccaagaa gtgtgcagat tccaacccga 1440
gaggggtgag cgcctaccta agccggccca gcccgttcga cctgttcatc cgcaagtcgc 1500

ccacgatcac ctgtctggtg gtggacctgg cacccagcaa ggggaccgtg aacctgacct 1560
ggtcccgggc cagtgggaag cctgtgaacc actccaccag aaaggaggag aagcagcgca 1620
atggcacgtt aaccgtcacg tccaccctgc cggtgggcac ccgagactgg atcgaggggg 1680
agacctacca gtgcagggtg acccaccccc acctgcccag ggccctcatg cggtccacga 1740
ccaagaccag cggcccgcgt gctgccccgg aagtctatgc gtttgcgacg ccggagtggc 1800
cggggagccg ggacaagcgc accctcgcct gcctgatcca gaacttcatg cctgaggaca 1860
tctcggtgca gtggctgcac aacgaggtgc agctcccgga cgcccggcac agcacgacgc 1920
agccccgcaa gaccaagggc tccggcttct tcgtcttcag ccgcctggag gtgaccaggg 1980
ccgaatggga gcagaaagat gagttcatct gccgtgcagt ccatgaggca gcgagcccct 2040
cacagaccgt ccagcgagcg gtgtctgtaa atcccggtaa atgagagctc 2090

<210> 7
<211> 945
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA

<400> 7
atggcttgca attgtcagtt gatgcaggat acaccactcc tcaagtttcc atgtccaagg 60
ctcattcttc tctttgtgct gctgattcgt ctttcacaag tgtcttcaga tgttgatgaa 120
caactgtcca agtcagtgaa agataaggta ttgctgcctt gccgttacaa ctctccgcat 180
gaagatgagt ctgaagaccg aatctactgg caaaaacatg acaaagtggt gctgtctgtc 240
attgctggga aactaaaagt gtggcccgag tataagaacc ggactttata tgacaacact 300
acctactctc ttatcatcct gggcctggtc ctttcagacc ggggcacata cagctgtgtc 360
gttcaaaaga aggaaagagg aacgtatgaa gttaaacact tggctttagt aaagttgtcc 420
atcaaagctg acttctctac ccccaacata actgagtctg gaaacccatc tgcagacact 480
aaaaggatta cctgctttgc ttccgggggt ttcccaaagc ctcgcttctc ttggttggaa 540
aatggaagag aattacctgg catcaatacg acaatttccc aggatcctga atctgaattg 600
tacaccatta gtagccaact agatttcaat acgactcgca accacaccat taagtgtctc 660
attaaatatg gagatgctca cgtgtcagag gacttcacct gggaaaaacc cccagaagac 720
cctcctgata gcaagcccgg gggtggtggg agcggtggtg gcggcagtgg cggcggcgga 780
actagtaata gtgactctga atgtcccctg tcccacgatg ggtactgcct ccatgatggt 840
gtgtgcatgt atattgaagc attggacaag tatgcatgca actgtgttgt tggctacatc 900
ggggagcgat gtcagtaccg agacctgaag tggtgggaac tgcgc 945

<210> 8
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 8
ctagttccgc cgccgccact gccgccacca ccgctcccac caccccc 47

<210> 9
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 9
ctcgaattcc accatggctt gcaattgtca gttgatgc 38

<210> 10
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 10
ctccccgggc ttgctatcag gagggtcttc 30

<210> 11
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 11
ctcactagtg aggtccagct tcagcagtc 29

<210> 12
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 12
ctcgcggccg cttaccgttt gatttccagc ttggtgcctc cacc 44

<210> 13
<211> 87
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 13
ctagactcga gccaccatgg gatggagctg tatcatcctc ttcttggtag caacagctac 60
aggtgtccac tccgaggtcc agctgca 87

<210> 14
<211> 79
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 14
gctggacctc ggagtggaca cctgtagctg ttgctaccaa gaagaggatg atacagctcc 60
atcccatggt ggctcgagt 79

<210> 15
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 15
gtccagctgc agcagtctgg 20

<210> 16
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 16
cgtttgattt caagcttggt gc 22

<210> 17
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 17
gcgcaagctt gaaatcaaac gggcctccac caagggccca 40

<210> 18
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 18
gcgcctcgag tcatttaccc ggagacaggg 30

<210> 19
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 19
gcgcaagctt gaaatcaaac gggcctccac acagagccca 40

<210> 20
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 20
gcgcctcgag tcatttaccg ggatttacag a 31

<210> 21
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 21
ggactagtaa tagtgactct gaatgtccc 29

<210> 22
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 22
attagcggcc gcttagcgca gttcccacca cttc 34

<210> 23
<211> 68
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 23
aagcttccac catgggatgg agctgtatca tcctcttctt ggtagcaaca gctacaggtg 60
tccactcc 68

<210> 24
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SYNTHETIC
OLIGONUCLEOTIDE

<400> 24
gggggtggtg ggagcggtgg tggcggcagt ggcggcggcg gaa 43